I concur with this review. M. Serabian 7/28/15

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Cellular, Tissue and Gene Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch

BLA NUMBER:	STN #125518.000
DATE PHARM/TOX MODULE RECEIVED BY CENTER:	13-December-2013
DATE REVIEW COMPLETED:	19-January-2015; amended 02-Jun-2015
PRODUCT:	Imlygic® (Talimogene laherparepvec, T-VEC, OncoVEX^{GM-CSF})
SPONSOR:	Amgen, Inc.
PROPOSED INDICATION:	Oncolytic immunotherapy for the treatment of regionally or distantly metastatic melanoma that is injectable
PHARM/TOX REVIEWER:	Ying Huang, Ph.D.
PHARM/TOX TEAM LEAD:	Alex Bailey, Ph.D.
PHARM/TOX SUPERVISOR:	Mercedes Serabian, M.S., DABT
DIVISION DIRECTOR:	Wilson Bryan, M.D.
OFFICE DIRECTOR:	Celia Witten, Ph.D., M.D.
PROJECT MANAGER:	Mark Davidson, RHIA

Formulation and Chemistry:
Imlygic® (Talimogene laherparepvec, T-VEC, OncoVEX^{GM-CSF}) is an attenuated version of the wild type (wt) herpes simplex virus type-1 (HSV-1) genome engineered to express human granulocyte macrophage colony-stimulating factor (hGM-CSF). This product is generated by modifying two regions of the wt HSV-1 new isolate JS: 1) deletion of the ICP34.5 and ICP47 genes and 2) incorporation of the hGM-CSF expression cassette into the ICP34.5 loci. T-VEC is supplied as a sterile, single use, preservative-free frozen liquid in a cyclic olefin polymer (COP) plastic resin vial for intralesional injection. Each vial contains 1.0 mL deliverable volume at a nominal potency of 10^6 plaque forming units (PFU)/mL or 10^8 PFU/mL. The drug product is supplied in 2 mL vials, each containing a recoverable product volume of 1 mL, and is stored at -80°C ±10°C until use. Once thawed, T-VEC is formulated in diluent buffer [redacted] sodium chloride as [redacted] and [redacted] myo-inositol as [redacted] prior to administration.

Abbreviations:

ATCC	American Tissue Culture Collection
BHK	Baby hamster kidney
COP	cyclic olefin polymer
CPE	Cell Pathological Effect
DRG	Dorsal root ganglia

ECACC	European Collection of Cell Culture (also European Collection of Animal Cell Cuture)
[redacted]	[redacted]
GD	Gestation day
GM-CSF	Granulocyte Macrophage Colony-Stimulating Factor
[redacted]	[redacted]
HSV-1	Herpes Simplex Virus type 1
ICP34.5	neurovirulence gene encoded by HSV-1
ICP47	antigen processing inhibitor encoded by HSV-1
IFN	interferon
IL	interleukin
i.c.	intracranial
i.p.	intraperitoneal
i.t.	intratumoral
i.v.	intravenous
MHC	major histocompatibility complex
MOI	Multiplicity of Infection
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
oHSV-1	oncolytic HSV-1
PBS	Phosphate Buffered Saline
PFU	Plaque Forming Unit
PKR	protein kinase R
[redacted]	[redacted]
s.c.	subcutaneous
TK	Toxicokinetic
T-VEC	Talimogene laherparepvec
US11	RNA binding protein encoded by HSV-1, which inhibits activation of cellular PKR
US12	ICP47 coding sequence

Application History:
13-December-2013: Pharmacology/Toxicology modules (Modules 2.4, 2.6, and 4) submitted
01-May-2014 – Product (CMC) module submitted
28-July-2014: Clinical module submitted
26-November-2014: Major CMC amendment[1]

Related file:
IND #12412; BioVex, Inc.; Herpes Simplex Type-1 Virus Encoded with Human Granulocyte-Macrophage Colony-Stimulating Factor (rHSV-1hGM-CSF); Oncolytic Vaccine (OncoVex) for treatment of solid tumors including malignant melanoma; ACTIVE

[1] Response to FDA questions - Facility and CMC areas

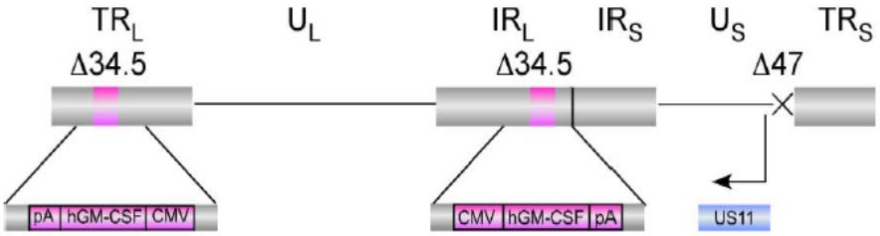
Note: BioVex Ltd, London, UK is the sponsor of IND #12412. Amgen, Inc. acquired all BioVex operations, including T-VEC, in 2011.

Table of Contents

INTRODUCTION

T-VEC is an attenuated replication competent oncolytic herpes simplex virus type-1 (oHSV-1) that has been genetically modified to constitutively express human GM-CSF. T-VEC was derived from a novel primary HSV-1 isolate (JS1, ECACC Accession Number 01010209) that demonstrates enhanced oncolytic activity towards tumor cells, as compared to the commonly used laboratory strains (e.g., 17syn+) and other primary isolates (Liu et al., 2003). To produce talimogene laherparepvec, the JS1 strain (a wt clinical isolate of HSV-1) was genetically modified by deleting the virulence genes that code for ICP34.5 and ICP47. Elimination of the ICP34.5 gene from JS1reduces neurovirulence by 10,000- to 1,000,000-fold, as compared to wt HSV-1 *(4, 5).* Wild type HSV-1 contains two copies of the gene for ICP34.5, and both copies were functionally deleted in talimogene laherparepvec by inserting two copies of human GM-CSF gene sequences. Deletion of the ICP47 gene also resulted in converting the HSV-1 late gene US11 into an immediate early gene, under the ICP47 promoter (Cassady et al., 1998). This modification results in increased Major Histocompatibility Complex (MHC) class I presentation *(6)*, thus permits proper antigen processing for both virus and tumor antigens, and is intended to aid in the generation of a T-cell mediated adaptive immune response.

The genomic structure of T-VEC is illustrated in the figure below:



The talimogene laherparepvec genome is shown with the positions of the ICP34.5 and ICP47 deletions marked as Δ34.5 and Δ47, respectively; immediate early expression of US11 is driven by the ICP47 promoter. The site of the hGM-CSF cassette insertion is shown in pink and expanded to show the composition of the hGM-CSF expression cassette; the cytomegalovirus (CMV) promoter, hGM-CSF cDNA and a bovine growth hormone polyadenylation signal (pA) signal.

The purported mechanisms of action (MOAs) of T-VEC (Figure 1) consist of: 1) lysis of the injected tumor, with subsequent release of viral particles that infect and destroy adjacent tumor cells and 2) release of tumor associated antigens (TAAs) as a result of tumor cell lysis, eliciting an immune response that is enhanced by the expressed GM-CSF to achieve a systemic anti-tumor effect. This tumor- selective oHSV is expected to have minimal adverse effects on normal tissues and a low risk of neurovirulence (compared to wild-type [wt] HSV-1).

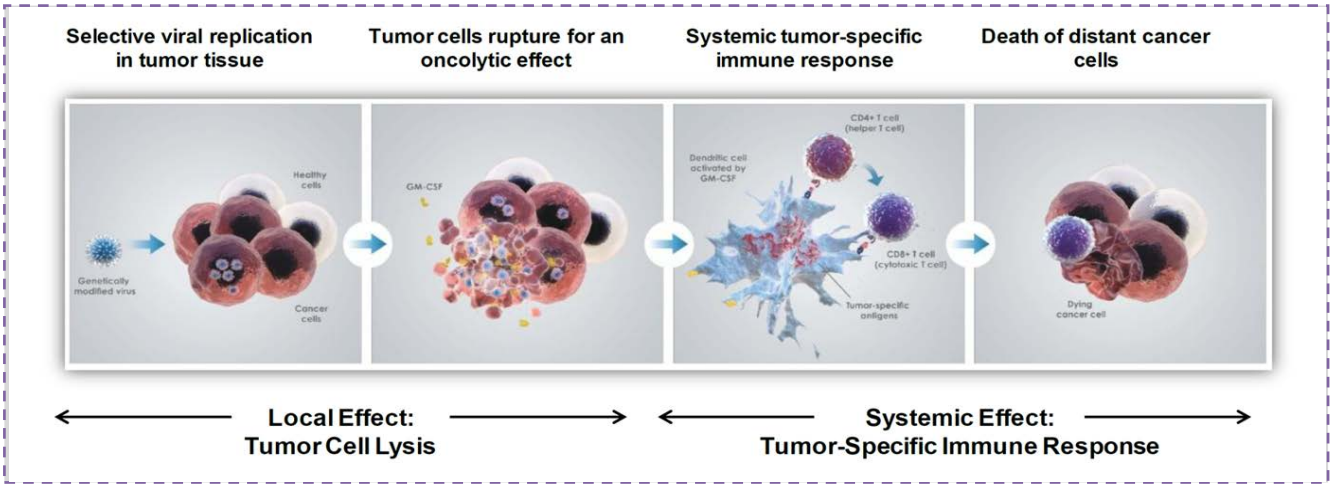


Figure 1. Purported MOAs of T-VEC

The sponsor--proposed label indication is: *"treatment of melanoma that is regionally or distantly metastatic"*. T-VEC will be administered by intralesional injection into cutaneous, subcutaneous, and nodal lesions. The recommended dose level is up to a maximum of 4 mL of T-VEC at a concentration of 10^8 PFU/mL. Subsequent administrations consist of up to 4 mL at a concentration of 10^8 PFU/mL. The proposed dosing schedule currently depicted in the label for T-VEC is shown in Table 1.

Table 1: Recommended Dosing Schedule for Imlygic

Treatment	Treatment interval	Maximum injection volume	Dose concentration	Prioritization of lesions to be injected
Initial	-	Up to 4 mL	10^6 (1 million) PFU/mL	• Inject largest lesion(s) first. • Prioritize injection of remaining lesion(s) based on lesion size until maximum injection volume is reached.
Second	3 weeks after initial treatment	Up to 4 mL	10^8 (100 million) PFU/mL	• Inject any new lesion(s) (lesions that may have developed since initial treatment) first. • Prioritize injection of remaining lesion(s) based on lesion size until maximum injection volume is reached.
All subsequent treatments (including re-initiation)	2 weeks after previous treatment	Up to 4 mL	10^8 (100 million) PFU/mL	• Inject any new lesion(s) (lesions that may have developed since previous treatment) first. • Prioritize injection of remaining lesions based on lesion size until maximum injection volume is reached.

According to the proposed label, the volume of T-VEC to be injected into each lesion is dependent on the size of the lesion and should be determined according to Table 2.

Table 2: Selection of Imlygic Injection Volume Based on Lesion Size

Lesion size (longest dimension)	Imlygic injection volume	Dose concentration: 10^6 (1 million) PFU/mL	Dose concentration: 10^8 (100 million) PFU/mL
> 5 cm	up to 4 mL	up to 4 million PFU	up to 400 million PFU
> 2.5 cm to 5 cm	up to 2 mL	up to 2 million PFU	up to 200 million PFU
> 1.5 cm to 2.5 cm	up to 1 mL	up to 1 million PFU	up to 100 million PFU
> 0.5 cm to 1.5 cm	up to 0.5 mL	up to 500,000 PFU	up to 50 million PFU
≤ 0.5 cm	up to 0.1 mL	up to 100,000 PFU	up to 10 million PFU

References:

1. Russell SJ, Peng KW, Bell JC. Oncolytic virotherapy. Nat Biotechnol. 30(7):658-70, 2012
2. Melcher A, Parato K, Rooney CM, Bell JC. Thunder and Lighting: Immunotherapy and Oncolytic Virus Collide. Mol Ther. 19(6):1008-16, 2011.
3. Toda M, Rabkin SD, Kojima H, Martuza RL. Herpes simplex virus as an in situ cancer vaccine for the induction of specific anti-tumor immunity. Human Gene Ther. 10:385-93, 1999.
4. Chou J, Kern ER. Mapping of herpes simplex virus-1 neurovirulence to gamma 134.5, a gene nonessential for growth in culture. Science 250(4985):1262-6, 1990.
5. Bolovan CA, Sawtell NM, Thompson RL. ICP34.5 mutants of herpes simplex virus type 1 strain 17syn+ are attenuated for neurovirulence in mice and for replication in confluent primary mouse embryo cell cultures. J Virol. 68(1):48-55, 1994.
6. Todo T, Martuza RL, Rabkin SD, Johnson PA. 2001. Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. PNAS. 98:6396-6401, 2001.
7. Mohr I and Gluzman Y. A herpesvirus genetic element which affects translation in the absence of the viral GADD34 function. EMBO J. 15(17):4759, 1996.
8. Mohr I, Sternberg D, Ward S, Leib D, Mulvey M, Gluzman Y. A herpes simplex virus type 1 γ34.5 second-site suppressor mutant that exhibits enhanced growth in culture glioblastoma cells is selectively attenuated in animals. J Virol. 75(11):5189-96, 2001.

Preclinical Studies

Numerous preclinical studies to evaluate the bioactivity and toxicity profiles of T-VEC were conducted. Many of the preclinical studies were performed under the sponsorship of BioVex during the early stages of product development, thus many of the study reports were issued by BioVex. The names T-VEC and OncoVEX$^{GM\text{-}CSF}$ are used interchangeably. In addition, various viral strains and constructs were used in some preclinical studies during product development; the construct names and their relationship to the final drug product are provided in Table 1.

Table 1. Viral Strain and Construct Nomenclature for Products used in the Preclinical Studies

Name	Other names that appear in some reports	Description
17*syn*+	17+	Serially passaged laboratory strain of wt HSV-1
JS1		Newly isolated strain of wt HSV-1
17*syn*+/34.5-/CMV-	17+/34.5-/CMV 17+/ICP34.5- 17+/34.5-	17*syn*+ with ICP34.5 deleted, containing the
JS1/34.5-/CMV-	JS1/34.5-	JS1 with ICP34.5 deleted, containing the
JS1/34.5-/47-	OncoVEX backbone, JS1/34.5-/47-/pA-	JS1 with ICP34.5 and ICP47 deleted
JS1/34.5-/47-/pA+		JS1 with ICP34.5 and ICP47 deleted, without US11 upregulation (US11 is separated from the ICP47 regulatory sequences by an artificially introduced polyA)
OncoVEX$^{mGM\text{-}CSF}$ OncoVEX$^{muGM\text{-}CSF}$ OncoVEX$^{mouseGM\text{-}CSF}$	JS1/34.5-/47-/$^{mGM\text{-}CSF}$ JS1/34.5-/47-/$^{muGM\text{-}CSF}$ JS1/34.5-/47-/$^{mouseGM\text{-}CSF}$	JS1 with ICP34.5 and ICP47 deleted, containing the gene encoding mouse GM- CSF. **This virus was used in preclinical studies in mice.**
Talimogene laherparepvec (United States Adopted Name (USAN))		
OncoVEX$^{GM\text{-}CSF}$ OncoVEX$^{hGM\text{-}CSF}$ OncoVEX$^{huGM\text{-}CSF}$ OncoVEX$^{humanGM\text{-}CSF}$	JS1/34.5-/47-/pA-/GM-CSF JS1/34.5-/47-/hGM-CSF JS1/34.5-/47-/huGM-CSF JS1/34.5-/47-/humanGM-CSF	JS1 with ICP34.5 and ICP47 deleted, containing the gene encoding human GM- CSF. **This virus was used in all clinical trials.**

Summary List of Pharmacology Studies

The following pharmacology studies were conducted under the sponsorship of BioVex to support the rationale for the administration of T-VEC in clinical trials. All listed studies are summarized in this review memo.

In Vitro Studies:

1. The Role of Upregulated US 11 Expression on the Anti-Tumor Effect of JS1/34.5-/47- in the HT29 Xenograft Tumor Model (Study #4648-00075)
2. Effect of ICP47 on Expression of Class I MHC on the Surface of Cells Infected with JS1-related viruses (Study #4848-00072)

3. A Comparative Assessment of 17*syn*+, JS1 and OncoVEX^{GM-CSF} Growth Property in [redacted] Cell Lines Permissive and Non-Permissive to Wild Type HSV-1 (Study #4648-00063)
4. Assessment of the *In Vitro* Lysis of Human Tumor Cells by OncoVEX^{GM-CSF} Virus using a [redacted] (Study #4648-00035)
5. Assessment of the Cytotoxic Effect of OncoVEX^{GM-CSF} Virus on Human Colorectal Cancer Cell Lines *in vitro* (Study #4648-00036)
6. Assessment of the Cytotoxic Effect of OncoVEX^{GM-CSF} Virus on Human Pancreatic Cancer Cell Lines *in vitro* (Study #4648-00037)
7. Assessment of the Cytotoxic Effect of OncoVEX^{GM-CSF} Virus on Human and Mouse Lung Cancer Cell Lines *in vitro* (Study #4648-00038)
8. Assessment of the *in vitro* Lysis of Human and Rat Prostate Tumor Cells by OncoVEX^{GM-CSF} Virus using a [redacted] (Study #4648-00055)

In Vivo Studies in Human Xenograft Tumor Models:

9. Assessment of the Safety and Anti-Tumor Effect of OncoVEX Viruses on Human Colorectal Carcinoma (HT-29) - Induced Tumors in BALB/c Nude Mice (Study #4648-00040)
10. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Human Glioblastoma Astrocytoma (U-87 MG) Induced Tumors in BALB/c Nude Mice (Study #4648-00064)
11. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Human Squamous Cell Carcinoma (Pharynx), FaDu Induced Tumors in BALB/c Nude Mice (Study #4648-00065)

In Vivo Studies in Syngeneic Tumor Models:

12. Cellular Mediated Immune Response in A20 Tumor Bearing Mice Treated with OncoVEX (Study #4648-00071)
13. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma (A20) - Induced Tumors in BALB/c Mice (Study #4648-00002)
14. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma (A20) - Induced Tumors in BALB/c Mice (Study #4648-00051)
15. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma (A20) - Induced Tumors in BALB/c Mice (Study #4648-00005)
16. Assessment of the Anti-Tumor Effect of OncoVEX Viruses in Mouse Colon Carcinoma (CT26) - Induced Tumors in BALB/c Mice (Study #4648-00008)
17. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma, A20 - Induced Tumors in BALB/c Mice (Study #4648-00006)
18. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma, A20 - Induced Tumors in BALB/c Mice (Study #4648-00013)
19. Assessment of the Effect of Repeat-Dose Intratumoral Administration of OncoVEX^{GM-CSF} in Tumor-Bearing BALB/c Mice (Study #4648-00012)
20. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma, A20 - Induced Tumors in BALB/c Mice (Study #4648-00039)

21. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma, A20 - Induced Tumors in BALB/c Mice Previously Exposed to Wild Type HSV-1 (Study #4648-00003)
22. Assessment of the Safety and Anti-Tumor Efficacy of OncoVEX Viruses in Immunosuppressed BALB/c Mice Bearing A20 - Induced Tumors (Study #4648-00011)
23. Measurement of CT26-specific Cytotoxic T lymphocytes after Intra-tumoral Injection of OncoVEX$^{mGM\text{-}CSF}$ into TT26 Tumor Bearing Female BALB/c Mice (Study #R20130079)
24. Comparison of the Anti-Tumor Effect of [redacted] Batches of OncoVEX$^{GM\text{-}CSF}$ in Mouse Reticulum Cell Sarcoma (A20) in BALB/c Mice (Study #4648-00018)
25. Comparison of the Anti-Tumor Effect of [redacted] Batches of OncoVEX$^{GM\text{-}CSF}$ produced by [redacted], with Evaluation of OncoVEX$^{mouseGM\text{-}CSF}$ produced by [redacted] in Mouse Reticulum Cell Sarcoma (A20) in BALB/c Mice (Study #4648-00034)
26. Comparison of the Anti-Tumor Effect of OncoVEX$^{GM\text{-}CSF}$ and OncoVEX$^{mouseGM\text{-}CSF}$ in Mouse Reticulum Cell Sarcoma (A20) in BALB/c Mice (Study #4648-00001)
27. Assessment of the Anti-Tumor Effect of OncoVEX$^{GM\text{-}CSF}$ in Combination with Radiation on CT26 Colon Carcinoma Tumors in BALB/c Mice (Study #4648-00019)
28. Non-GLP Toxicology Study - Assessment of the Effect of Administration of OncoVEX$^{GM\text{-}CSF}$ in Combination with Arimidex in A20 Tumor-Bearing Mice (Study #4648-00020)
29. Assessment of the Anti-Tumor Effect and Safety of OncoVEXGM-CSF in Combination with [redacted] on CT26 Colon Carcinoma Tumors in BALB/c Mice (Study #4648-00025)

Other In Vivo Studies:

30. Assessment of the Anti-tumor Effect of Two HSV-1/ICP34.5 Deleted Viruses (17+/34.5-/CMV[redacted] and JS1/34.5-/CMV[redacted] strains) on Human Colorectal Carcinoma (HT29)-Induced Tumors in BALB/c Nude Mice (Study #4648-00066)
31. Assessment of the Anti-tumor Effect of Two HSV-1/ICP34.5 Deleted Viruses (17+/34.5-/CMV[redacted] and JS1/34.5-/CMV-[redacted] strains) on Human Breast Adenocarcinoma (MDA MB231)-Induced Tumors in BALB/c Nude Mice (Study #4648-00067)
32. Assessment of the Anti-tumor Effect of Two HSV-1/ICP34.5 Deleted Viruses (17+/34.5-/CMV-[redacted] and JS1/34.5-/CMV-[redacted] strains) on Human Glioblastoma Astrocytoma (U-87MG)-Induced Tumors in BALB/c Nude Mice (Study #4648-00068)
33. Assessment of the Anti-tumor Effect of Two HSV-1/ICP34.5 Deleted Viruses (17+/34.5-/CMV-[redacted] and JS1/34.5-/CMV-[redacted] strains) on Human Squamous Cell Carcinoma (Pharynx) FaDu-Induced Tumors in BALB/c Nude Mice (Study #4648-00069)

Pharmacology Studies

Note: The designated 'Study #' for each study was assigned by the current sponsor, Amgen. The year that is listed in the identifier information provided with the study title is the year when the study report was issued by Amgen

In Vitro Studies:

1. **The Role of Upregulated US 11 Expression on the Anti-Tumor Effect of JS1/34.5-/47- in the HT29 Xenograft Tumor Model,** Study# 4648-00075; conducted by BioVex Ltd.; non-GLP; 2012

   Objective: To assess the *in vivo* anti-tumor effect of JS1/34.5-/47-/pA (US11 under the control of the ICP47 regulatory sequence), compared to JS1/34.5-/47-/pA+.

   [redacted]

   Results (Figure 1):

   - [redacted]
   - [redacted]

- 

Reference:

1. Liu BL, Robinson M, Han ZQ, Branston RH, English C, Reay P, McGrath Y, Thomas SK, Thornton M, Bullock P, Love CA, and Coffin RS. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumor properties. Gene Ther. 10(4):292-303, 2003.

2. 

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References:

1. Hill AB1, Barnett BC, McMichael AJ, and McGeoch DJ. HLA class I molecules are not transported to the cell surface in cells infected with herpes simplex virus types 1 and 2. J Immunol. 152(6):2736-41, 1994.
2. York IA1, Roop C, Andrews DW, Riddell SR, Graham FL, Johnson DC. A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8+ T lymphocytes. Cell. 77(4):525-35, 1994.
3. Garcia-Lora A, Algarra I, and Garrido F. MHC class I antigens, immune surveillance, and tumor immune escape. J Cell Physiol. 195(3):346-55, 2003.

4. Boon T1, Coulie PG, Van den Eynde BJ, and van der Bruggen P. Human T cell responses against melanoma. Annu Rev Immunol. 24:175-208, 2006

3. **A Comparative Assessment of 17*syn*+, JS1 and OncoVEX^{GM-CSF} Growth Property in [redacted] Cell Lines Permissive and Non-Permissive to Wild Type HSV-1**, Study #4648-00063; conducted by BioVex Ltd.; non-GLP; 2012

   Objective: To evaluate the growth property of OncoVEX^{GM-CSF} compared with wild type strains, JS1 and 17*syn*+.

- [redacted]
- [redacted]

[redacted]

[redacted]

Reference:

1. Montgomery RI1, Warner MS, Lum BJ, and Spear PG. Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. Cell. 87:3):427-36, 1996.

4. **Assessment of the *In Vitro* Lysis of Human Tumor Cells by OncoVEX^{GM-CSF} Virus using a** [redacted], Study #4648-00035; conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

- [redacted]
- [redacted]

[redacted]**sed**

[redacted]

5. **Assessment of the Cytotoxic Effect of OncoVEX^{GM-CSF} Virus on Human Colorectal Cancer Cell Lines *in vitro***, Study #4648-00036; conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

[redacted]

6. **Assessment of the Cytotoxic Effect of OncoVEX^{GM-CSF} Virus on Human Pancreatic Cancer Cell Lines *in vitro***, Study #4648-00037); conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

7. **Assessment of the Cytotoxic Effect of OncoVEX^{GM-CSF} Virus on Human and Mouse Lung Cancer Cell Lines *in vitro***, Study # 4648-00038; conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

8. **Assessment of the *in vitro* Lysis of Human and Rat Prostate Tumor Cells by OncoVEX^{GM-CSF} Virus using a [redacted],** Study #4648-00055; conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

- [redacted]
- [redacted]
- [redacted]

[redacted]

[redacted]

[redacted]

In Vivo Studies in Human Xenograft Tumor Models:

9. Assessment of the Safety and Anti-Tumor Effect of OncoVEX Viruses on Human Colorectal Carcinoma (HT-29) - Induced Tumors in BALB/c Nude Mice, Study #4648-00040; conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

[redacted]

[redacted]

[redacted]

- [redacted]
- [redacted]

[redacted]

10. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Human Glioblastoma Astrocytoma (U-87 MG) Induced Tumors in BALB/c Nude Mice, Study #4648-00064; conducted by BioVex Ltd.; non-GLP, 2010

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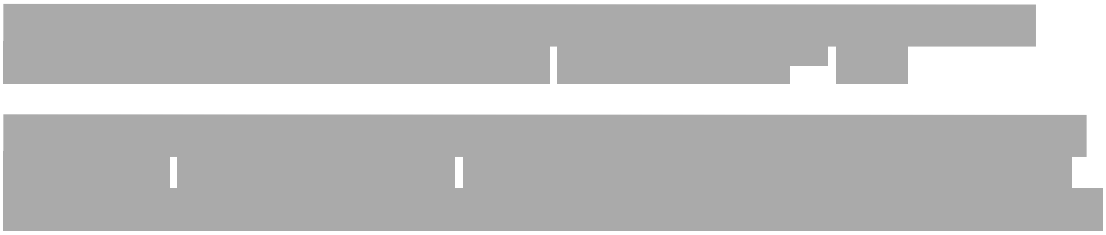
11. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Human Squamous Cell Carcinoma (Pharynx), FaDu Induced Tumors in BALB/c Nude Mice, Study #4648-00065; conducted by BioVex Ltd.; non-GLP, 2010

In Vivo Studies in Syngeneic Tumor Models:

12. Cellular Mediated Immune Response in A20 Tumor Bearing Mice Treated with OncoVEX, Study #4648-00071; conducted by BioVex Ltd; non-GLP, 2012



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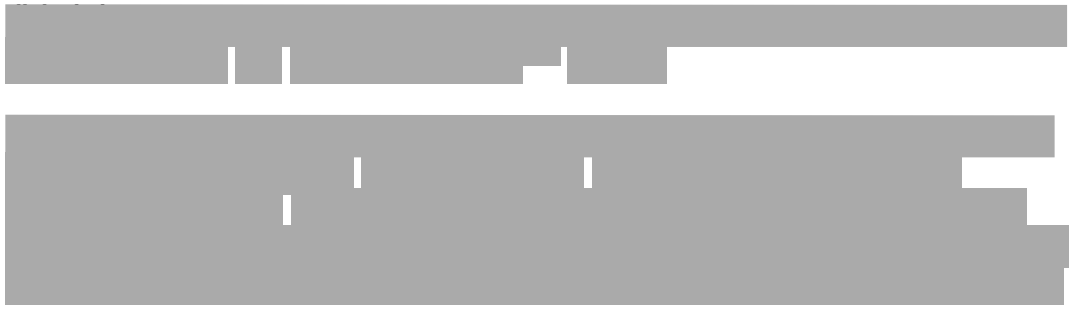
13. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma (A20) - Induced Tumors in BALB/c Mice (4648-00002)



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14. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma (A20) - Induced Tumors in BALB/c Mice, Study #4648-00051; conducted by BioVex Ltd.; non-GLP, 2010

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[redacted]

15. **Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma (A20) - Induced Tumors in BALB/c Mice,** Study #4648-00005; conducted by BioVex Ltd.; non-GLP, 2010

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- [redacted]
- [redacted]

[redacted]

16. **Assessment of the Anti-Tumor Effect of OncoVEX Viruses in Mouse Colon Carcinoma (CT26) - Induced Tumors in BALB/c Mice**, Study #4648-00008; conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

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[redacted]

17. **Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma, A20 - Induced Tumors in BALB/c Mice**, Study #4648-00006; conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

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18. **Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma, A20 - Induced Tumors in BALB/c Mice**, Study #4648-00013; conducted by BioVex Ltd.; non-GLP, 2010

(b) (4)

19. Document No.: 4648-00012, October 18, 2010; non-GLP
Title: Non-GLP Toxicology Study – Assessment of the Effect of Repeat-Dose Intratumoral Administration of OncoVEX^{GM-CSF} in Tumor-Bearing BALB/c Mice
Objective: To evaluate the safety of repeat i.t. injection of OncoVEX^{GM-CSF} in an A20 mouse tumor model.
Testing Facility: BioVex Ltd.; gross pathology and histopathology were conducted by

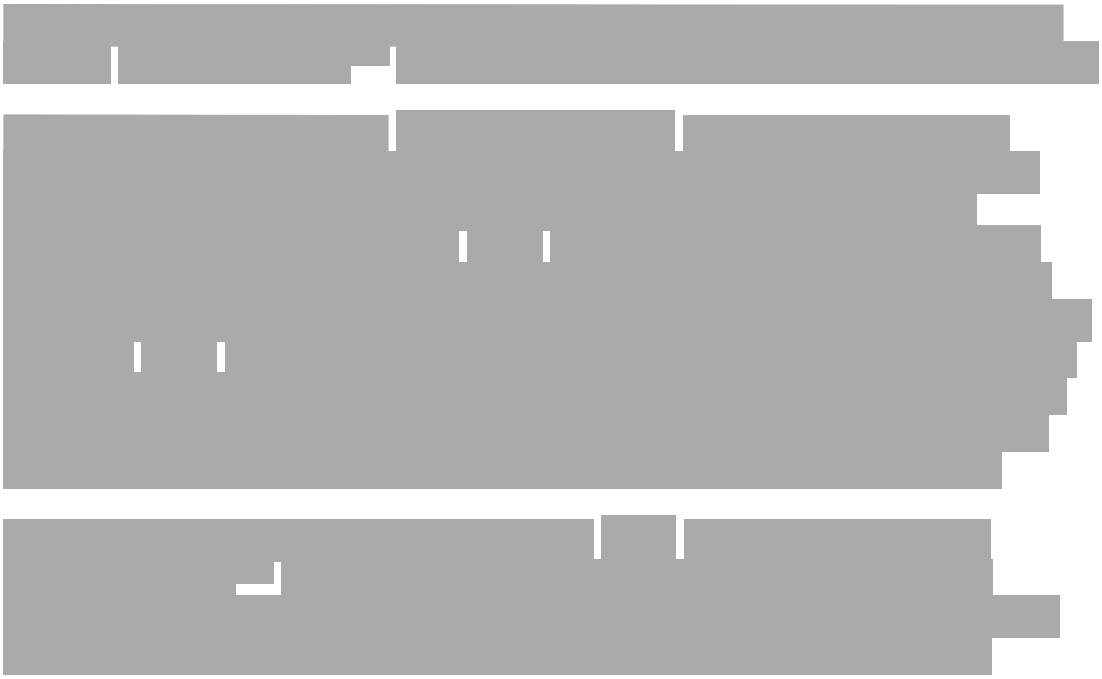
1 page determined to be not releasable: (b)(4)

[redacted]

20. **Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma, A20 - Induced Tumors in BALB/c Mice**, Study # 4648-00039; conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

21. Assessment of the Anti-Tumor Effect of OncoVEX Viruses on Mouse Reticulum Cell Sarcoma, A20 - Induced Tumors in BALB/c Mice Previously Exposed to Wild Type HSV-1, Study #4648-00003; conducted by BioVex Ltd.; non-GLP, 2010



(b) (4)

22. Assessment of the Safety and Anti-Tumor Efficacy of OncoVEX Viruses in Immunosuppressed BALB/c Mice Bearing A20 - Induced Tumors, Study #4648-00011; conducted by BioVex Ltd.; non-GLP, 2010

1 page determined to be not releasable: (b)(4)

- [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

23. **Measurement of CT26-specific Cytotoxic T lymphocytes after Intra-tumoral Injection of OncoVEX$^{mGM-CSF}$ into TT26 Tumor Bearing Female BALB/c Mice,** Study #R20130079; conducted by Amgen Inc.; non-GLP, 2013

[redacted]

[redacted]

[redacted]

2 pages determined to be not releasable: (b)(4)

[redacted]

24. Comparison of the Anti-Tumor Effect of [redacted] Batches of OncoVEX^{GM-CSF} in Mouse Reticulum Cell Sarcoma (A20) in BALB/c Mice, Study #4648-00018; conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

1 page determined to be not releasable: (b)(4)

[redacted]

[redacted]

25. **Comparison of the Anti-Tumor Effect of [redacted] Batches of OncoVEX^{GM-CSF} produced by [redacted], with Evaluation of OncoVEX$^{mouseGM-CSF}$ produced by [redacted] in Mouse Reticulum Cell Sarcoma (A20) in BALB/c Mice**, Study #4648-00034; conducted by BioVex Ltd.; non-GLP, 2010

[redacted]

[redacted]

- [redacted]
- [redacted]

2 pages determined to be not releasable: (b)(4)

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26. Comparison of the Anti-Tumor Effect of OncoVEX$^{GM\text{-}CSF}$ and OncoVEX$^{mouseGM\text{-}CSF}$ in Mouse Reticulum Cell Sarcoma (A20) in BALB/c Mice, Study #4648-00001; conducted by BioVex Ltd.; non-GLP, 2010

1 page determined to be not releasable: (b)(4)

27. Assessment of the Anti-Tumor Effect of OncoVEX^{GM-CSF} in Combination with Radiation on CT26 Colon Carcinoma Tumors in BALB/c Mice, Study #4648-00019; conducted by The non-GLP, 2010. Necropsies were conducted at Histopathology was conducted at by a board-certified veterinary pathologist

28. Non-GLP Toxicology Study - Assessment of the Effect of Administration of OncoVEX^{GM-CSF} in Combination with Arimidex in A20 Tumor-Bearing Mice, Study #4648-00020; conducted by BioVex Ltd.; non-GLP, 2010. Necropsies were conducted at BioVex by technicians from Histopathology was conducted at by a board-certified veterinary pathologist

1 page determined to be not releasable: (b)(4)

- [redacted]

[redacted]

Other In Vivo Studies:

29. Assessment of the Anti-Tumor Effect and Safety of OncoVEX^{GM-CSF} in Combination with [redacted] on CT26 Colon Carcinoma Tumors in BALB/c Mice, Study #4648-00025; conducted by BioVex Ltd.; non-GLP, 2013. Necropsies were conducted at BioVex by technicians from [redacted]. Histopathology was conducted at [redacted] by a board-certified veterinary pathologist.

[redacted]

2 pages determined to be not releasable: (b)(4)

30. Assessment of the Anti-tumor Effect of Two HSV-1/ICP34.5 Deleted Viruses (*17*+/34.5-/CMV- and JS1/34.5-/CMV- strains) on Human Colorectal Carcinoma (HT29)-Induced Tumors in BALB/c Nude Mice, Study #4648-00066; conducted by BioVex Ltd.; non-GLP, 2011

31. Assessment of the Anti-tumor Effect of Two HSV-1/ICP34.5 Deleted Viruses (17+/34.5-/CMV- and JS1/34.5-/CMV- strains) on Human Breast Adenocarcinoma (MDA MB231)-Induced Tumors in BALB/c Nude Mice, Study #4648-00067, conducted by BioVex Ltd.; non-GLP, 2011

(b) (4)

32. Assessment of the Anti-tumor Effect of Two HSV-1/ICP34.5 Deleted Viruses (17+/34.5-/CMV- and JS1/34.5-/CMV- strains) on Human Glioblastoma Astrocytoma (U-87MG)-Induced Tumors in BALB/c Nude Mice, Study #4648-00068, conducted by BioVex Ltd.; non-GLP, 2011

Objective: To compare the anti-tumor activity of two HSV-1/ICP34.5 deleted viruses, 17+/34.5-/CMV- and JS1/34.5-/CMV-, in a murine human U-87MG tumor xenograft model.

33. Assessment of the Anti-tumor Effect of Two HSV-1/ICP34.5 Deleted Viruses (17+/34.5-/CMV- and JS1/34.5-/CMV strains) on Human Squamous Cell Carcinoma (Pharynx) FaDu-Induced Tumors in BALB/c Nude Mice, Study #4648-00069, conducted by BioVex Ltd.; non-GLP, 2011

(b) (4)

Summary List of Biodistribution (BD) Studies

The following biodistribution (BD) studies were conducted to evaluate viral DNA levels and human GM-CSF transcript levels following *in vivo* administration of T-VEC in animals. The studies were conducted under the sponsorship of BioVex or Amgen.

1. OncoVEX^{GM-CSF}: Single Dose Biodistribution Study in the Mouse with an 84-Day Observation Period (Document No. 4648-00030)
2. Talimogene Laherparepvec: Intratumoral Biodistribution Study in the BALB/c Mouse (Document No. 115857)
3. Assessment of the Extent and Duration of Leakage of JS1/34.5-/CMV [redacted] Virus Following Intratumoral Injection into CT26 Tumors in BALB/c Mice (Document No. 4648-00010)
4. Assessment of In Vivo GM-CSF Levels after Intratumoral Injection with OncoVEX in Mouse Reticulum Cell Sarcoma (A20) in BALB/c Mice (Document No. 4648-00016)

Studies #5-8 listed below included both safety and BD parameters. Detailed summaries of these studies are provided in the Toxicology section of this review memo.

5. OncoVEX^{GM-CSF}: Repeat Dose Toxicity and QPCR Biodistribution Study in the Mouse Followed by a 24-hour, 28 or 56-Day Observation Period (Document No. 4648-00027)
6. OncoVEX^{GM-CSF}: Repeat Dose Toxicity and QPCR Biodistribution Study in the Mouse Followed by a 24-hour, 4 or 12 Week Observation Period (Document No. 4648-00028)
7. OncoVEX^{GM-CSF}: A Single Dose Toxicity Study in Male Dogs with Administration into the Prostate (Document No. 4648-00032)
8. Talimogene Laherparepvec: Intravenous Embryo-Fetal Development Study in the Balb/c Mouse (Document No.117250)

Biodistribution (BD) Studies

Note: The term 'Document No.' is the Amgen document number.

1. **Document No.:** 4648-00030
   Study Report No.: , conducted in compliance with GLP
   Report Date: December 03, 2003
   Title: OncoVEX^{GM-CSF}: Single Dose Biodistribution Study in the Mouse with an 84-Day Observation Period
   Objective: To assess virus biodistribution (BD) following s.c. and i.v. administration of OncoVEX^{GM-CSF}.
   Testing Facility: .

   Test and Control Articles:
   - Test - OncoVEX^{GM-CSF}, Batch No. 1.6x10^8 PFU/mL
   - Control - Vehicle

   Study Animals:
   - BALB/c mice (55/sex), 41-57 days old, 17.9-23.9 g for males and 16.5-20.9 g for females; purchased from
   - Animals were assigned to study groups using a computerized blocking procedure designed to achieve body weight balance with respect to group. At the time of randomization, the weight variation of the animals was within two standard deviations of the mean body weight for each sex.

   Study Design:
   - Study groups (15 mice/sex/group):
     - Group 1 - vehicle control (s.c.)
     - Group 2 - OncoVEX^{GM-CSF}, 0.6x10^7 PFU/mouse, s.c. into the flank
     - Group 3 - OncoVEX^{GM-CSF}, 0.6x10^7 PFU/mouse, i.v. into the tail vein

     Dose volume - 100 µL/mouse; animals injected on day 1

- Sacrifice time points: 3 mice/sex/group were sacrificed at 24 hours and 14, 28, 56, and 85 days post-injection

- Evaluations:
  - Clinical observations twice daily for mortality and moribundity; once weekly for abnormal signs
  - Injection site - irritation (erythema and edema) scoring on days 1 and 2, weekly thereafter according to a [redacted].
  - Body weights - days -1 and 1, and weekly thereafter
  - Food consumption - individual food consumption recorded weekly
  - Urine collection - animals were placed into urine collection cages starting within 5 minutes after dosing, where they remained up to 18 hours post-injection; and prior to the day 56 sacrifice, urine was analyzed via qPCR for viral DNA levels

- qPCR analysis:
  - Blood samples were collected and processed for qPCR analysis for HSV DNA levels and for anti-HSV antibodies titers via [redacted] (conducted by [redacted], BioVex Study No. 4648-00074; non-GLP study)
  - Tissues - testes and epididymides/ovaries, spleen, liver, kidneys, heart, lungs, eyes, brain, trigeminal ganglia, and injection site were collected and processed for qPCR analysis. The sciatic nerve from the injection site of found dead animals was also collected.
  - qPCR assay sensitivity was ≥64 copies/µg DNA (lower limit of quantitation [LLOQ], calculated from the qPCR assay detection of 6.4 copies/reaction in a background of 0.1, 0.5 and 1.0 µg murine DNA per reaction in Assay Validation Report #4648-00056a, [redacted]

Comments:

- Per the 2006 CBER guidance document titled '*Guidance for Industry: Gene Therapy Clinical Trials-Observing Subjects for Delayed Adverse Events'* (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm072957.htm; [accessed 5.03.15), an assay sensitivity of ≤50 copies/µg gDNA is recommended. Although the qPCR assay sensitivity is slightly higher than this level, since the study was conducted prior to release of this guidance, and the difference is small, the assay sensitivity is acceptable.

- Dose analysis of the prepared test article (Appendix 8 of the study report) lists a concentration of $0.6x10^8$ PFU/mL, thus the administered dose level is consistent with the target dose level. The identity of the test article was also confirmed.

Results:

- Two Group 2 males were found dead on days 13 and 15. Clinical signs included hunched, hypoactive, limited use of hind limbs, recumbent, and cold to touch. No macroscopic lesions were observed for either animal.

- Clinical observations:
  - Limited use of the injected limb for one Group 2 animal on days 13 and 15.
  - Sores/scabs on the abdomen or tails of Groups 1 and 3 animals (similar incidence).

- Injection site reaction:
  - Slight edema at the s.c. injection site in two Group 2 males at 7 days post-injection.
  - Slight erythema at the s.c. injection site in one Group 1 female at 1 day post-injection.

- There were no significant differences in mean body weights among study groups.

- There were no significant differences in mean food consumption values among study groups.

- qPCR analysis following s.c. injection (Group 2):
  - At 24 hours post-injection:
    - Viral DNA at the injection site: <64 - 8,190 copies/µg gDNA and in the urine: 1/6 females, 68.6 copies/µg gDNA (the only animal with enough urine to analyze). No viral DNA was detected in any other tissues evaluated.

  - At 14 days post-injection:
    - Urine samples from the 14-day sacrifice cohort show 1/6 samples negative, 2/6 samples below the LLOQ, and 3/6 samples (1/3 female and 2/3 males) were positive (169.1 copies/µg gDNA).
    - Viral DNA in the testes of 1/3 males (102.5 copies/µg gDNA) and at the injection site of 1/3 females (151.6 copies/µg gDNA).
    - Blood and all other tissues were negative.

  - At 28 days post-injection, no viral DNA was detected in any samples.

- qPCR analysis following i.v. injection (Group 3):
  - At 24 hours post-injection:
    - Viral DNA was detected in all tissues evaluated in 3/3 males and 1/3 females.

- The primary sites of localization were the blood (29,369 - 80,216 copies/µg DNA), injection site (402 - 18,995 copies/µg DNA) and liver (2,334 - 10,855 copies/µg DNA).
- Viral DNA was also detected in the spleen (523.8 - 1,705 copies/µg/DNA), heart (272.5 - 2443 copies/µg DNA), lungs (125.9 - 961 copies/µg DNA), eyes, kidney, trigeminal ganglia, gonads and urine (<64 - 508 copies/µg DNA); and brain (<LLOQ).

▪ The other 2/3 females high levels of viral DNA at the injection site (186,008 and 68,478 copies/µg DNA) only.

Note: These data suggest that the test article was likely not administered correctly.

▪ HSV DNA was not detected in urine samples from 2 females and 3 males, with <LLOQ in 1/3 females.

o At 14 days post-injection:

▪ The DNA levels were significantly decreased as compared to the 24-hour levels: blood was the primary site of localization in 3 mice/sex (8,410-44,879 copies/µg DNA), followed by the heart in 4/6 mice (74.9-866.4 copies/µg DNA), lungs (<64-512.3 copies/µg DNA), injection site (2/6 mice were negative for viral DNA, 1/6 were below the LLOQ, and 3/6 contained 106-6,111.3 copies/µg DNA), and the kidneys, trigeminal ganglia, liver and spleen (≤355.3 copies/µg DNA).

▪ Eyes (2/3 males and 1/3 females), testes (1/3 males), brain (2/3 females), injection site (1 mouse/sex), trigeminal ganglia (1/3 females) were all negative. The remaining eye and testes samples were <LLOQ, and 1/6 brain samples was 76.5 copies/µg DNA.

o At 28 days post-injection:

▪ The DNA levels continued to decrease as compared to the day 14 levels: the highest levels were in the blood in 3 mice/sex (4,944-63,091 copies/µg DNA), followed by injection site (1/6 samples at 2,652 copies/µg DN; samples for 5/6 mice were contaminated with PCR inhibitors), heart (<64-2492 copies/µg DNA), liver, lungs, spleen and trigeminal ganglia (≤511copies/µg DNA), and eyes, gonads, kidney, and brain (≤ LLOQ).

o At 56 days post-injection:

▪ Low DNA levels (<LLOQ) were detected in the blood (3/6 mice), liver (5/6 mice), spleen (6/6 mice), and trigeminal ganglia and heart (1/6 mice each). Blood samples from 2/6 mice had ≤118.6 copies/µg DNA.

Comment:

- The BD data indicate minimal clearance of the injected HSV DNA levels in the blood and urine.

- Anti-HSV antibody analysis:
  - All mice injected with OncoVEX^{GM-CSF} sero-converted, displaying levels of 1-100 antibody index values for the day 28 samples (index value = 0.2 in the control mice).
  - The i.v. injected mice (Group 3) had higher antibody levels at each time point (days 28, 56, and 85) compared to the s.c. injected mice (Group 2). The antibody levels for the i.v. injected mice were approximately 2-fold higher than the s.c. injected mice at each time point.
  - Antibody levels in both OncoVEX^{GM-CSF} injected groups continued to increase up to the last time point (85 days post-injection).

- Macroscopic findings (liver mass, small testis, and fluid in the abdominal cavity of a Group 1 female) were considered incidental.

Report Conclusion: Following s.c. injection of OncoVEX^{GM-CSF} to mice, viral DNA was detected primarily at the injection site, with systemic clearance nearly complete by 28 days post-injection. Following i.v. injection, viral DNA was detected at the injection site and in the blood, with clearance from all tissues (except blood) by 56 days.

2. **Document No.:** 115857
   Study Report No.: 8261471; non-GLP
   Report Date: April 23, 2013
   Title: Talimogene Laherparepvec: Intratumoral Biodistribution Study in the BALB/c Mouse
   Objective: To evaluate virus biodistribution (BD) following intratumoral (i.t.) administration of OncoVEX^{GM-CSF}
   Testing Facility:
   Test and Control Articles:
   - Test - OncoVEX^{GM-CSF} (talimogene laherparepvec), Lot No. BP1047HA, titer $1x10^8$ PFU/mL
   - Control - sodium chloride, sorbitol and myo-inositol

   Study Animals:
   - BALB/c mice, 23-25 g; supplier not provided.
   - A20 murine B cell lymphoma/suspension culture (200 µL; supplied by was s.c. injected into the right flank at $2x10^5$ cells/mouse. The report did not provide the randomization method for the study groups.

   Study Design:
   - When group mean tumor volumes reached approximately 100 mm^3 (about 12 days post-injection), tumors were injected with OncoVEX^{GM-CSF} at dose levels listed in Table 1 below, at a dose volume of 50µL/mouse (designated as study

day [SD] 1). Product administration was repeated on SDs 4 and 7. Animals were sacrificed at approximately 24 hours (SD 8, n=5 mice/sex/group), 7 days (SD 14, n=5-9 mice/sex/group), and 84 days (SD 91, remaining mice) after last test or control article administration , or were euthanized for humane reasons (when the size of the tumor caused impairment or when tumors became ulcerated, generally ≥3,500 mm^3).

- Urine collection from all the animals – prior to sacrifice, animals were placed into urine collection cages for up to 18 hours.
- Blood collection from all the animals at sacrifice.
- The following tissues were collected from all animals: eye, bone marrow (femur), heart, kidney, liver, lung, lymph nodes, spleen, blood, injection site (tumor), gonads, brain (thick cross section including portions of brain stem, cerebellum, and cerebrum), lachrymal glands, salivary glands, feces (from the rectum), and nasal mucosa.

Comment:

- The sponsor did not provide a rationale for the dosing schedule and the sacrifice time points. However, this reviewer considers these design factors to be acceptable based on the BD and clearance profiles following single s.c. administration in non-tumor bearding mice (Document No.: 4648-00030)

Table 1. Study Design

Group	Number of Animals	Dose (PFU/mouse)	Scheduled Euthanasia (Study Days)
1	14 (Female)	Vehicle[a]	8, 14
2	14 (Male)	Vehicle[a]	8, 14
3	24 (Female)	1 x 10^5	8, 14, 91
4	24 (Male)	1 x 10^5	8, 14, 91
5	24 (Female)	5 x 10^5	8, 14, 91
6	24 (Male)	5 x 10^5	8, 14, 91

(b) (4) sodium chloride, sorbitol, and myo-inositol,

- Evaluations:
  - Moribund animals were sacrificed and all tissue samples described above were collected
  - Animals found dead before their scheduled sacrifice were discarded without evaluation
  - Clinical observations - twice daily for mortality and moribundity
  - Behavioral observations - biweekly
  - Body weights - biweekly
  - Tumor volumes (mm^3, V=0.536 × L × W) - biweekly

- qPCR Analysis:
  - Tissue samples were frozen and shipped to for qPCR analysis

- qPCR assay sensitivity was ≥64 copies/μg DNA (calculated 6.4 copies/reaction) in a background of 0.1, 0.5 and 1.0 μg murine DNA per reaction (assay validation Report #4648-00056a)

Results:

- Unscheduled deaths:
  - For humane reasons, no control animals were maintained on study after SD 14. A total of 7/24 (Group 3), 6/24 (Group 4), 1/24 (Group 5) and 12/24 (Group 6) mice died prior to their scheduled sacrifice.

Comment:

- All Group 6 males died prior to SD 56, while the majority of the Group 6 females survived to this interval. The report did not discuss this finding. However the unscheduled death rates were similar in males as compared to females in the low dose groups (Group 4 and Group 3), thus, a consistent gender effect was not shown.

- Clinical and behavioral observations and body weights:
  - No markedly different behavior was observed in Groups 3-6 as compared to control Groups 1-2.
  - One Group 4 male exhibited hunched posture on SD 56 and one Group 5 female mouse had a ulceration at the tumor site on SD 70.
  - There was no significant difference in mean body weights between the virus and control groups.

- Tumor volume:
  - There were no significant differences in mean tumor volumes between the virus and control groups up to SDs 10 and 12 for the male and female groups, respectively.
  - Decreases in mean tumor volumes after SDs 10/12 were observed across Groups 3-6. The decreases were not associated with gender or viral dose levels (Tables 8 and 9 of the report).

- qPCR analysis (Appendix 1 of the report):
  - The [redacted] validation study data show a limit of detection (LOD) of 45.7 copies/reaction (equivalent to a C_T value of 35.87 [numbers of cycles required for the [redacted] to across the threshold]) indicating moderate amounts of target nucleic acid.

    Note: The LLOQ, established at a C_T value of 36.19, was considered equivalent to the LOD.

  - Viral DNA was not detected in bone marrow, eyes, lachrymal glands, nasal mucosa, or feces.

  - A total of 95% of virus injected tumors was positive on SD 8, 45% on SD 14 and 20% on SD 91, indicating a time-dependent clearance profile. The

average copy number/μg (mean ±SD) was approximately 3-fold higher in Groups 5 and 6 mice than in Groups 3 and 4 mice; there appeared to be an inverse relationship between tumor volume and viral copy number.

- A total of 13% of the blood samples from virus injected animals were positive across all time points, with 15% on SD 8, 13% on SD 14, and 6% after SD 14.

- The liver, lymph node, and spleen for Groups 3-6 mice were positive through SD 91.

- Viral DNA was detected in the brain in one mouse each in Group 3 (SD 58) and Group 4 (SD 91). These animals did not have detectable viral DNA in the blood or tumor. The sponsor stated that this finding was related to the tropism of HSV-1 for nervous system tissues.

- Viral DNA was detected in the gonads of one Group 3 mouse (SD 58) and in the salivary gland of one Group 4 mouse (SD 91). Each mouse also had relatively high blood viral DNA levels.

- Viral DNA was also detected in the heart (n=4), kidney (n=3), and lung (n=3) among the four animals with the highest blood viral DNA levels. The sponsor stated that the presence of viral DNA in these tissues was likely a result of blood perfusion to these tissues.

Report Conclusion: The BD of talimogene laherparepvec following i.t. injection was predominantly restricted to the tumor and blood, and to tissues likely associated with immune-mediated viral clearance (spleen, lymph node, liver). The absence of viral DNA in the lachrymal glands, nasal mucosa or feces indicates a low likelihood of secondary exposure (i.e., shedding) of viral DNA from tears, mucous, or feces. Viral DNA found in the brains of two virus-injected mice was not associated with adverse effects in these animals.

Comments:

- The [redacted] validation report (Appendix 1 of the study report) did not specify the amount of input gDNA (μg) thus, the value of 45.7 copies/reaction does not represent for the sensitivity in copies/μg gDNA. Per the validation report, the LOD/LLOQ C_T value of 35.87 was comparable to the previous validated data. Thus, this reviewer assumes that the qPCR assay is of reasonable sensitivity.

- The qPCR inhibition test was not conducted on the urine samples.

3. **Document No.:** 4648-00010; non-GLP
Title: Assessment of the Extent and Duration of Leakage of JS1/34.5-/CMV [redacted] Virus Following Intratumoral Injection into CT26 Tumors in BALB/c Mice
Objective: To assess the amount and duration of 'leakage' of JS1/34.5-/CMV [redacted] following a single i.t. injection in CT26 xenografts in BALB/c mice.
Testing Facility: BioVex Ltd.
Test and Control Articles:
   - Test - JS1/34.5-/CMV-[redacted], Batch No. [redacted]
   - Control - [redacted]

   Methods:
   - Five female BALB/c mice (4-6 weeks old, 16-20 g) were purchased from [redacted]. The mice were s.c. injected with $2x10^6$ CT26 tumor cells into the right flank on day -7. On day 0 the mice were i.t. injected with JS1/34.5-/CMV-[redacted] at 5 x 10^6 PFU/mouse (50 µL). On day 8 fine needle aspirates (FNA) of the tumor were collected, followed by swabbing of the punctured sites on days 1-4 and 9-11. Plaque assays (detecting <1 x 10^2 PFU) were performed on all swabs and the aspirate using the [redacted]; the test article served as a positive control for the assay.

   Results: No plaques were detected from any of the collected samples.

Report Conclusion: The results indicate that there was no evidence of any virus 'leakage' from the tumor FNA biopsy sites.

4. **Document No.:** 4648-00016; non-GLP
Title: Assessment of *in Vivo* GM-CSF Levels after Intratumoral Injection with OncoVEX in Mouse Reticulum Cell Sarcoma (A20) in BALB/c Mice
Objective: To assess tumor and serum levels of GM-CSF after i.t. injection of OncoVEX^{GM-CSF}.
Testing Facility: BioVex Ltd.
Test and Control Articles:
   - Test - OncoVEX^{GM-CSF}, Batch No. [redacted]; OncoVEX backbone JS1/34.5-/47-, Batch No. [redacted]; JS1/34.5-/CMV[redacted], Batch No. [redacted]
   - Control - [redacted]

   Methods:
   Thirty five female BALB/c mice (4-6 weeks old, 16-20 g) were purchased from [redacted]. The mice were s.c. injected with 2 x 10^6 murine reticulum cell sarcoma (A20)/mouse into the right flank. Ten days later (day 0) the mice were i.t. injected (50 µL) with $5x10^6$ PFU/animal of OncoVEX^{GM-CSF} (Group 1; 12 mice), OncoVEX backbone (Group 2; 12 mice), or vehicle control (Group 3; 6 mice). Tumor and blood samples were collected from one Group 3 mouse/sacrifice time point and two Groups 1-2 mic/sacrifice time point at days 1 (24 hours post-injection), 4, 7, 15, and

22. The GM-CSF levels were determined by [redacted].

Results:

- Mean huGM-CSF levels (0.55 μg of huGM-CSF/mg of total protein) were detected in the tumor in Groups 1-2 at day 1. The levels decreased 10-fold by day 4 (0.03 μg huGM-CSF/mg of total protein). By day 7, mean huGM-CSF levels in the tumors decreased another 10-fold (0.006 μg huGM-CSF/mg of total protein). No huGM-CSF was detected in the tumors at day 15. No huGM-CSF was detected at any time points in the tumors injected with OncoVEX backbone virus.

- Mean serum huGM-CSF levels in Groups 1-2 were 0.00015 μg huGM-CSF/mg of total protein on day 1, which were several orders of magnitude less than the corresponding tumor samples. No serum huGM-CSF was detected at later time points. No serum huGM-CSF was detected at any time points from animals injected with OncoVEX backbone virus.

Report Conclusion: The results suggest that following a single i.t. injection of OncoVEX$^{GM\text{-}CSF}$ in A20 tumors in BALB/c mice, huGM-CSF was detected in the solid tumor and in the blood at 24 hours post-injection. No huGM-CSF was detected in serum on day 4; however, huGM-CSF was detected in the tumors up to day 7.

Comments:

- Measurable levels of hGM-CSF were detected in the tumors, but not in blood, following intratumoral injection of OncoVEX$^{GM\text{-}CSF}$ to tumor-bearing mice (Document No. 4468-00016). However, limited data on the presence of GM-CSF in the blood following i.v. injection of OncoVEX$^{mouseGM\text{-}CSF}$ in the mice were provided.

- Measurable levels of hGM-CSF were not detected in blood in subjects following intratumoral injection of OncoVEX$^{GM\text{-}CSF}$. Thus potential systemic exposure to hGM-CSF in humans is low, indicating low/minimal risk for systemic and reproductive toxicity.

References:

1. Wilson JG. Methods for administering agents and detecting malformations in experimental animals. In: Wilson JG, Warkany J, editors. *Teratology: principles and techniques*. Chicago (IL): University of Chicago Press; 1965. p. 262-77.

2. Dawson, AB. A note on the staining of the skeleton of cleared specimens with alizarin red S. Stain Technol. 1:123-124, 1926.

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of T-VEC following administration in various animal species. The studies were conducted under the sponsorship of BioVex or Amgen.

Toxicology Studies:

1. Assessment of Effect of Subcutaneous Administration of OncoVEX Viruses In Male and Female BALB/c Mice (Document No. 4648-00007)
2. OncoVEX$^{GM\text{-}CSF}$: Repeat Dose Toxicity and Biodistribution Study in the Mouse with a 56 day Observation Period (Document No. 4648-00026)
3. OncoVEX$^{GM\text{-}CSF}$: Repeat Dose Toxicity and QPCR Biodistribution Study in the Mouse Followed by a 24-hour, 28 or 56-Day Observation Period (Document No. 4648-00027)
4. OncoVEX$^{GM\text{-}CSF}$: Repeat Dose Toxicity and QPCR Biodistribution Study in the Mouse Followed by a 24-hour, 4 or 12 Week Observation Period (Document No. 4648-00028)
5. OncoVEXGM-CSF Repeat Dose Toxicity in the Mouse Followed by a 24 Hour, or 4 Week Observation Period (4648-00029)
6. OncoVEX$^{GM\text{-}CSF}$: A Single Dose Toxicity Study in Male Dogs with Administration into the Prostate (Document No. 4648-00032)

Toxicology Studies Designed to Evaluate Mechanism of Toxicity:

7. The Effect of Acyclovir on the Replication of OncoVEX$^{GM\text{-}CSF}$ (Document No. 4648-00024)
8. Assessment of the Effect of Repeat-Dose Subcutaneous Administration of OncoVEXmouse$^{GM\text{-}CSF}$ Viruses in Female BALB/c Mice (Document No. 4648-00052)
9. Assessment of Neurovirulence Following Intranasal Administration of OncoVEX$^{GM\text{-}CSF}$ in Female BALB/c Mice (Document No. 4648-00014)
10. Assessment of Effect of Intra Cerebral Administration of OncoVEX Viruses in Female BALB/c Mice (Document No. 4648-00004)
11. A Comparative Assessment of 17*syn+* and OncoVEX Virus Reactivation from Latency in the Peripheral Nervous System of BALB/c Mice (Document No. 4648-00015)

Developmental and Reproductive Toxicology Studies:

12. Talimogene Laherparepvec: Intravenous Embryo-Fetal Development Study in the BALB/c Mouse (Document No. 117250)

Other Toxicology Studies:

13. Talimogene laherparepvec (, OncoVEX^{GM-CSF}): Tolerability and Anti-Tumor Effects on Human Colorectal Carcinoma (HT-29) Tumors in mice and BALB/c nude mice
14. Talimogene laherparepvec : Pathology Report for, "T -VEC efficacy in Sarcoma Xenograft Tumor Model" Intrahepatic Arterial Route to Rats
15. GLP Toxicity Study of HSV vector OncoVEXGM-CSF Administered by the Intrahepatic Arterial Route to Rats

Toxicology Studies

Note: The term 'Document No.' is the Amgen document number.

Toxicology Studies:

1. **Document No.:** 4648-00007, November 05, 2010; non-GLP
   Title: Assessment of Effect of Subcutaneous Administration of OncoVEX Viruses in Male and Female BALB/c Mice
   Objective: To evaluate the safety of OncoVEX^{GM-CSF} and OncoVEX$^{mouseGM-CSF}$ following repeat s.c. injection.
   Testing Facility: BioVex Ltd.; gross pathology and histopathology were conducted by

   Test Articles:
   - Test - OncoVEX^{GM-CSF}, Batch No. , titer 1x10^8 PFU/mL; OncoVEX$^{mouseGM-CSF}$ Batch No. , titer 1x10^8

   Study Animals:
   - A total of 60 BALB/c mice/sex (supplier, age, body weights not provided)

   Study Design:
   - Study groups (20 mice/sex/group):
     - Group 1 - OncoVEX^{GM-CSF}, 1x10^7 PFU/mouse/injection
     - Group 2 - OncoVEX^{GM-CSF}, 1x10^6 PFU/mouse/injection
     - Group 3 - OncoVEX$^{mouseGM-CSF}$, 1x10^7 PFU/mouse/injection

   - Test and control articles (100 µL/mouse) were s.c. injected into the right flank of each animal on SDs 1, 4, 7, 10, and 13. Body weights were recorded prior to injection and on SDs 3, 7, 10, 14, 21, 28, 35, and 43, and animals were sacrificed on SD 43. Comprehensive gross pathology was conducted and histopathology was performed on the brain, heart, kidneys, liver, lungs, and spleen from Groups 1 and 3 (3 mice/sex/group).

Results:

- One Group 1 male was found dead on SD 14; the animal did not show abnormal clinical signs prior to death.

- No adverse clinical signs or significant differences in mean body weights were observed in all other mice.

- An enlarged spleen was observed in 1/10 males each in Groups 1 and 3; this finding correlated with the microscopic finding of extramedullary hematopoiesis.

- No other histopathology findings were reported in the tissues examined.

Report Conclusion: Healthy mice (both genders) that were administered multiple s.c. injections of OncoVEX$^{GM\text{-}CSF}$ or OncoVEX$^{mouseGM\text{-}CSF}$ did not display any difference in clinical signs, body weights, gross pathology, or histopathology (limited tissues) findings compared to the concurrent control group or to each other.

Comment:

- The sponsor did not provide a rationale for the dosing schedule and the sacrifice time points. However, this reviewer considers these intervals acceptable based on the BD and clearance profiles following single s.c. administration in non-tumor bearing mice (Document No. 4648-00030). The study data supported the dosing regimen of once every 2 weeks in the initial, early-phase clinical trial.

2. **Document No.:** 4648-00026
Study Report No.: 2040-003, conducted in compliance with GLP
Report Date: March 11, 2003
Title: OncoVEX$^{GM\text{-}CSF}$: Repeat Dose Toxicity and Biodistribution Study in the Mouse with a 56 day Observation Period
Objective: To evaluate the safety and BD profile of OncoVEX$^{GM\text{-}CSF}$ following single s.c. injection followed by a 30-day observation period, and five repeated s.c. injections followed by up to a 56-day observation period
Testing Facility:
Test and Control Articles:
    - Test - OncoVEX$^{GM\text{-}CSF}$, Batch No. , titer 2x10^8 PFU/mL
    - Control -
Study Animals:
    - Balb.c mice (81/sex), 49 days old, 16.8-24.0 g for males and 13.1-20.4 g for females; purchased from
    - Animals were assigned to groups using a set of random letter permutations

Study Design:

- Study groups for toxicology evaluation (6 mice/sex/sacrifice time point) and BD (3-6 mice/sex/sacrifice time point):
    - Group 1 - control
    - Group 2 - OncoVEX$^{GM\text{-}CSF}$, 1x10^5 PFU/mouse/injection (repeat tox)

- Group 3 - OncoVEX$^{GM\text{-}CSF,}$ $1x10^6$ PFU/mouse/injection (repeat tox)
- Group 4 - OncoVEX$^{GM\text{-}CSF,}$ $0.8x10^7$ PFU/mouse/injection (repeat tox and repeat BD)
- Group 5 - OncoVEX$^{GM\text{-}CSF,}$ $0.8x10^7$ PFU/mouse, (single tox)
- Group 6 - OncoVEX$^{murineGM\text{-}CSF}$, $0.8x10^7$ PFU/mouse (single tox)

- Test and control articles were s.c. injected (100 μL/mouse/injection), on SD 1 or SDs 1, 4, 7, 10, and 13

- Groups 1-4 (repeat injections) were sacrificed on SDs 14, 42, and 67 (the Group 4 SD 14 blood samples were not analyzed for clinical pathology due to shipping error). Groups 5-6 animals (single injection) were sacrificed on SDs 2 and 29/31.

- Evaluations:
  - Clinical observations – daily; mortality and moribundity - twice daily; physical examinations - weekly
  - Body weights - SDs -1 and 1, and weekly thereafter
  - Food consumption (by each cage) - weekly
  - Blood samples collected at sacrifice for hematology and clinical chemistry (each 3 mice/sex/group)(SD 29/31 Group 4 samples were collected but were not analyzed due to technical errors)
  - Urine samples collected overnight (16-18 hours) prior to sacrifice (SDs 2, 14, 29/31 and 41) and pooled for urinalysis Bone marrow smears collected for myelogram analysis
  - Comprehensive gross pathology (all animals) and histopathology on a comprehensive list of tissues (Groups 1, 4, 5, and 6), and unscheduled deaths
  - For the Group 4 BD animals, blood, urine, and tissues (testes/ epididymides, ovaries, spleen, liver, kidneys, heart, lungs, eyes, brain, trigeminal ganglia, and injection site) were collected on SDs 14 and 67 and SD 14 samples were processed for qPCR analysis of HSV DNA levels while SD 67 samples were not analyzed.

Note: Due to significant study deviations consisting of: 1) blood and urine sample collection failure at several time points, 2) failure to collect tissues from unscheduled deaths, 3) insufficient amount of test article to enable completion of dosing for Groups 4-6), 4) deprivation of food and water in some animals prior to sacrifice, the repeat injection portion of the study was repeated under Study No. 4648-00027 (#3 in the toxicology study List).

Results (Toxicology):

- There were a total of nine unscheduled deaths; 8/9 were found dead (no histopathology conducted):

Table 1. Unscheduled Deaths

Animal number	Group number	Day of death	Comment
24	2M	11	Found dead, cause of death not determined
97	2F	14	Found dead, partly cannibalized, cause of death not determined
102	2F	13	Found dead, cause of death not determined
110	3F	11	Sacrificed due to poor condition, cause of death not determined
118	4F	14	Found dead at necropsy, accidental death (hemorrhage in spinal cord)
137	4F (PCR)	22	Found dead, partially cannibalized. Tissues sampled for PCR analysis – macroscopic examination performed
149	5F	15	Found dead, partly autolysed, cause of death not determined
153	6F	2	Found dead, partly cannibalized, cause of death not determined
157	6F	29	Found dead, partly cannibalized, cause of death not determined

- For the remaining animals, there were no test article related clinical signs or significant differences in mean body weights, food consumption, or urinalysis values among the study groups.

- Due to various technical deviations, clinical pathology data were collected for the SD 42 samples only. No meaningful differences in mean hematology or clinical chemistry parameters were noted. A slightly higher proportion of eosinophils in the bone marrow smears were observed for Group 4 on SD 2, as compared to controls.

- Macroscopic and microscopic analyses (single injection – Groups 5-6):
    - At 24 hours post-injection (SD 2), there were no macroscopic findings attributed to the test articles. Minor fasciitis at the injection site of many Group 5 and 6 animals, characterized by inflammatory cell infiltration of the fascia of the subcutis, consisting mainly of neutrophils, but also lymphocytes and macrophages, with occasional necrotic debris was observed. The incidence and severity of the fasciitis was slightly greater in Group 6 compared to Group 5.
    - At 28 days post-injection (SD 29/31), a few Group 6 animals had a red focus at the injection site, which was diagnosed as dermatitis. Histopathology showed minor fasciitis at the injection site (1/6 males each in Groups 5 and 6), indicating recovery. Myositis/myopathy at the injection site was also observed.

- Macroscopic and microscopic analyses (repeat injections – Groups 2-4):
    - At 24 hours post-last injection (SD 14), there were no test article-related macroscopic findings, with the exception of a sore at the injection site in one Group 4 male. Microscopic findings seen the Group 4 only included:
        - Spleen – increased hematopoiesis in 6/6 males and 5/6 females

- Thymic atrophy in 6/6 males and 5/5 females
- Injection site fasciitis in 6/6 males and 5/5 females

- At 29 days post-last injection (SD 42), there were no macroscopic findings attributed to the test articles, except for a red focus at the injection site in a Group 4 male. Microscopic findings in the spleen and thymus were comparable to the control animals in incidence and severity. A total of 1/6 Group 4 males showed minor fasciitis at the injection site, and dermatitis and myositis/myopathy were present in a few animals across the groups.

Results (BD) - Group 4:

- The qPCR assay sensitivity was ≥64 copies/µg DNA (LLOQ, calculated from 6.4 copies/reaction in a background of 0.1, 0.5 and 1.0 µg murine DNA per reaction in Assay Validation Report #4648-00056,

- Blood samples were not analyzed due to a shipping error; urine samples collected at 8 days post-last injection were analyzed. At 24 hours post-last injection, viral DNA was detected in the injection sites of 1/3 mice (169.5 copies/µg g DNA) and in urine samples of 2/3 females (167.7 and 359.5 copies/µg gDNA). At 29 days post-last injection, no viral DNA was detected in any tissue samples. PCR inhibitor levels were detected in 1/6 injection site samples, (potentially a false negative).

Report Conclusion: Due to the numerous study deviations, the relevance of the resulting data is questionable.

3. **Document No.:** 4648-00027
   Study Report No.: 2024-009; conducted in compliance with GLP
   Report Date: February 6, 2002

Note: The study report also contains a follow-up Expert Panel Review Report issued by . on August 28, 2012, on the brain findings

Title: OncoVEX^{GM-CSF}: Repeat Dose Toxicity and QPCR Biodistribution Study in the Mouse Followed by a 24-hour, 28, or 56-Day Observation Period
Objective: To evaluate the safety and BD profile of OncoVEX^{GM-CSF} following five repeated s.c. administration followed by up to 56-day observation period
Testing Facility: .
Test and Control Articles:

- Test - OncoVEX^{GM-CSF}, Batch No. , titer 2x10^{8} PFU/mL
- Control -

Study Animals:

- BALB/c mice (69/sex), 70 days old, 23.7-28.9 g for males and 20.2-25.1 g for females; purchased from
- Animals were assigned to groups using a randomization by body weight.

Study Design:

- Study groups: Groups 1-5 for toxicology evaluation (6 mice/sex/group/sacrifice time point) and Groups 2 and 6 for BD assessment (3 mice/sex/group/sacrifice time point):
    - Groups 1 - 2 - [redacted] control
    - Group 3 - OncoVEX^{GM-CSF}, $1x10^5$ PFU/mouse/injection
    - Group 4 -OncoVEX^{GM-CSF}, $1x10^6$ PFU/mouse/injection
    - Group 5 -OncoVEX^{GM-CSF}, $1x10^7$ PFU/mouse/injection
    - Group 6 - OncoVEX^{GM-CSF}, $1x10^7$ PFU/mouse/injection

- Test and control articles were s.c. injected (100 μL/mouse) between the scapulae (scruff) of each animal. Each animal was injected on SDs 1, 4, 7, 10, and 13. Animals in Groups 1 and 3-5 were sacrificed at days 1 and 29 after the last injection (SDs 14 and 41) for toxicology evaluation and Groups 2 and 6 were sacrificed on SDs 14, 42, and 70 for BD assessment.

Comment:

- The sponsor did not provide a rationale for the dosing schedule and the sacrifice time points. However, this reviewer considers these intervals acceptable based on the BD and clearance profiles following single s.c. administration in non-tumor bearing mice (Document No. 4648-00030). The study data supported the dosing regimen of once every 2 weeks in the initial, early-phase clinical trial.

- Evaluations:
    - Clinical observations – daily; mortality and moribundity - twice daily; physical examinations - weekly
    - Body weights - SDs -1 and 1, and weekly thereafter
    - Food consumption (by cage; mice were group-housed) - weekly
    - Blood samples collected at sacrifice for clinical pathology and immune response (Groups 1-2, 3-5)
    - Bone marrow smears collected from Groups 1-5
    - Pooled urine samples collected overnight (16-18 hours) prior to sacrifice (Groups 1-5); individual urine samples collected for BD (Groups 2 and 6, on SDs 13-14, 14-15, 40-41, and 68-69)
    - Gross pathology and histopathology on comprehensive list of tissues from Groups 1 and 5, as well as unscheduled deaths
    - Histopathology on the spleen and brain of Groups 3-4 (all sacrifice intervals) and on the brain of Group 6 animals (SD 70 sacrifice)
    - BD - Group 6 - blood and tissues (testes/epididymides, ovaries, spleen, liver, kidneys, heart, lungs, eyes, trigeminal ganglia, duodenum, and injection site) were collected on SDs 14, 42, and 70 and processed for qPCR analysis of HSV DNA levels. The SD 70 brain samples were not analyzed. The qPCR assay sensitivity was ≥64 copies/μg DNA (LLOQ, calculated from 6.4 copies/reaction in a background of 0.1, 0.5 and 1.0 μg murine DNA per reaction [Assay Validation Report #4648-00056, [redacted]

Results (Toxicology):

- Unscheduled deaths:
  - One Group 6 female was sacrificed on SD 14 due to a compromised physical condition (body weight loss, hunched posture, swollen/blue abdomen, etc.). A distended gastro-intestinal tract was grossly observed; no histopathology examination was conducted.
  - On SD 22, one Group 3 female was sacrificed due to a subcutaneous tissue mass. Histopathology confirmed a spontaneous mammary adenocarcinoma which the pathologist considered unrelated to test article administration.

- There were no clinical signs that were related to test article administration.

- There were no significant differences in mean body weights or food consumption among the study groups.

- The mean hemoglobin level for Group 5 (SD 41) and mean total WBC counts for Group 4 (SD 42) were slightly decreased as compared to respective controls; however, since these changes occurred in only one group at a single time point, they were not considered to be of toxicological significance.

- There were no significant differences in mean clinical chemistry parameters or urinalysis values among the study groups.

- Macroscopic findings:
  - On SD 14, two Group 5 males had red injection sites and one Group 5 female had a large spleen that correlated to the microscopic findings.

- Microscopic findings:
  - On SD 14, all findings were in the Group 5 animals only. For example: 1) as compared to the control animals, a higher incidence of injection site reactions (fasciitis, dermatitis, acanthosis, inflammatory responses) and splenic findings (more prominent hematopoiesis in the red pulp) was observed; 2) one male had a focal encephalopathy; 3) perivascular gliosis with loss of neuropils was seen in the posterior cerebral hemisphere of one animal; 4) increased eosinophilia, vacuolation, and nuclear shrinkage (suggestive of early cortical necrosis) were observed in the anterior section of the cerebrum.

  - On SD 42, the microscopic findings were similar to those on SD 14; however, injection site fasciitis and the brain lesions were no longer evident, and the other findings were less frequent and less severe.

  - On SD 70, there were no lesions observed in the brain or other tissues examined.

Results (BD)

- SD 14 (24 hours post-last injection)
  - Viral DNA was detected in 5/6 injection site samples (84 - 29,473 copies/µg gDNA)
  - Viral DNA was detected at a low level (up to 98 copies/µg gDNA) in 2/6 blood samples. The viral DNA was also detected at a low level (up to 68 copies/µg gDNA) in 4/12 urine samples.
  - Viral DNA was not detected in any of the other tissues tested, suggesting that either the test article did not localize to these tissues or that the test article completely cleared within 24 hours following the last injection of test article.
  - In the animal sacrificed due to a deteriorating condition, viral DNA was detected in the brain (3,299 copies/µg gDNA), trigeminal ganglia (414 copies/µg gDNA), and duodenum (193 copies/µg gDNA); viral DNA levels were below the LLOQ of the assay in the spleen and injection site.

- SD 42 (28 days post-last injection)
  - Viral DNA was not detected in blood or in any tissues examined. Additional analysis of the injection site samples showed the presence of a qPCR inhibitor. When the samples were diluted in an attempt to dilute the inhibitor, negative results were still obtained, indicating a true negative.
  - SD 70 samples were not analyzed because of negative viral DNA levels at SD 42.

Report Conclusion: Subcutaneous administration of OncoVEX^{GM-CSF} in mice did not result in overt toxicity, with a no-observed-adverse-effect level (NOAEL) of $1x10^7$ PFU/mouse/injection. Viral DNA was detected at low levels in the injection site, blood and urine at 24 hours following the administration of the 5th injection of the product. Viral DNA was also detected at very low levels in urine samples collected between 1-41 hours following the 5th injection. There was no viral DNA detected in any of the other tissues tested on SDs 14-42, and SD70 samples were not analyzed.

Comments:

- A dose level of $1x10^7$ PFU/mouse/injection was 88-fold higher than the maximum human dose level (on a PFU/kg basis) administered in the first-in-human clinical trial (Document No. 4648-00028), and 70-fold higher than the recommended maximum dose level (on a PFU/kg basis) listed in the label for T-VEC.

- The expressed human GM-CSF is not bioactive in the mice, thus the findings are likely not related to exposure to this human protein.

4. **Document No.:** 4648-00028
   Study Report No.: 2024-026, conducted in compliance with GLP
   Report Date: December 06, 2004
   Title: OncoVEX^{GM-CSF}: Repeat Dose Toxicity and QPCR Biodistribution Study in the Mouse Followed by a 24-hour, 4, or 12 Week Observation Period

Objective: To evaluate the safety and BD profile of OncoVEX^{GM-CSF} following 12 (toxicology) or 5 (BD) repeated s.c. injections
Testing Facility:
Test and Control Articles:

- Test - OncoVEX^{GM-CSF}, Batch No. , titer 2x10^8 PFU/mL
- Control -

Study Animals:

- BALB/c mice (204 mice), 12-13 weeks old, 23.6-30.0 g for males and 19.2-25.9 g for females; purchased from
- Animals were assigned to groups using a randomization by body weight

Study Design:

- Study groups: Groups 1-4 for toxicology evaluation (6 mice/sex/sacrifice time point) and Groups 5-6 for BD assessment (5 mice/sex/sacrifice time point):
    - Group 1 - Vehicle control
    - Group 2 - OncoVEX^{GM-CSF}, 1x10^5 PFU/mouse/injection
    - Group 3 - OncoVEX^{GM-CSF}, 1x10^6 PFU/mouse/injection
    - Group 4 - OncoVEX$^{GM-CSF,}$ 1x10^7 PFU/mouse/injection
    - Group 5 - Vehicle control
    - Group 6 - OncoVEX$^{GM-CSF,}$ 1x10^7 PFU/mouse/injection

- Test and control articles (100 μL/mouse) were s.c. injected into the flank of each animal. Groups 1 to 4 (Toxicity phase) were injected once weekly for 12 weeks and were sacrificed at 24 hours and 4 and 12 weeks post-last injection. Groups 5 and 6 (BD phase) were injected once weekly for 5 weeks and were sacrificed at 24 hours and 4 and 12 weeks post-last injection. The first injection was administered on SD 1.

Comments:

- The sponsor did not provide a rationale for the dosing schedule and the sacrifice time points. However, this reviewer considers these intervals acceptable based on the BD and clearance profiles following single s.c. administration in non-tumor bearing mice (Document No. 4648-00030). The study data supported the dosing regimen of once every 2 weeks in the initial, early-phase clinical trial.

- The expressed human GM-CSF is not bioactive in the mice, thus the findings are likely not related to exposure to this human protein.

- Evaluations:
    - Clinical observations - daily; mortality and moribundity - twice daily; physical examinations - weekly
    - Body weights - SDs -1 and 1, and weekly thereafter
    - Food consumption (by cage) - weekly
    - Blood samples collected at sacrifice for clinical pathology and immune response (Groups 1-4)

- Bone marrow smears collected from Groups 1-4
- Pooled urine samples collected overnight (16-18 hours) prior to sacrifice (Groups 1-4); individual urine samples collected prior to sacrifice for BD (Groups 5-6)
- Gross pathology and histopathology on comprehensive list of tissues from Groups 1 and 4, as well as unscheduled deaths.
- Histopathology on the liver, spleen, and kidney of Groups 2-3 animals and histopathology on the injection sites of Groups 2-3 animals at 24 hours and 4 weeks. At 12 weeks, histopathology analysis was conducted on the injection site samples only.
- BD - Groups 5-6 - blood and tissues (testes/epididymides, ovaries, spleen, liver, kidneys, heart, lungs, eyes, brain, trigeminal ganglia, duodenum, and injection site) were collected at sacrifice and processed for qPCR analysis of HSV DNA levels.

Results (Toxicology):

- Unscheduled deaths:
  - One male and one female each in Groups 2 and 6 died during the first two weeks post-injection and were replaced. The report stated that the cause of death for these animals was possibly related to accidental intra-peritoneal injection of the test article
  - Four Group 1 animals died of 'various causes', one animal each in Groups 1 and 3 had lymphoreticular tumors; two Group 2 animals were found dead (undetermined cause); one Group 4 animal had hemorrhage in the brain.;
  - One animal each in Groups 1 and 4 exhibited convulsive episodes and died on the day of their scheduled sacrifice.
  - Macroscopic and microscopic findings in these animals were generally similar to those in animals surviving to terminal sacrifice.

- There were no significant differences in clinical observations, body weights, or food consumption among the study groups.

- At 4 weeks post-last injection, means total WBC, neutrophil, and lymphocyte counts were significantly increased in Groups 3 and 4 females compared to controls.

- Compared to controls: Bone marrow smears for the Group 4 males included decreased mean late erythroblast and total erythropoietic cell values, and increased mean myeloid/erythroid ratio values at 24 hours post-last injection. At 12 weeks post-last injection, increased mean total erythropoietic cells were observed for Group 4 males and females.

- There were no significant differences in mean clinical chemistry or urinalysis values among the study groups.

- There were no significant gross pathology findings among the study groups.

- Microscopic findings at the injection site in Groups 2-4:
  - At 24 hours post-last injection - fasciitis/fibrosis (more severe in females [not dose-dependent]; dose-dependent severity in males).
  - Dermatitis and myositis/myopathy in Group 4 males.
  - At 4 weeks post-last injection - reduced incidence of fibrosis and signs of healing.
  - At 12 weeks post-last injection - no microscopic findings

- There were no microscopic findings attributed to OncoVEX^{GM-CSF} in any other tissues examined from any animal.

- Anti-HSV-1 antibody analysis:
  - Results were reported as an antibody index (AI) in comparison with set of high low and intermediate control sera. Samples with an AI of <0.9 were considered to be negative, samples with an AI of ≥0 .9, but ≤1.1 were classified as equivocal, and samples with AI of >1 .1 were considered positive for antiHSV-1 IgG.
  - [redacted] were detected in all Groups 2-4 animals in a dose-dependency pattern, which persisted for the duration of the study.

Results (BD) [redacted]

- The qPCR assay sensitivity was ≥64 copies/µg DNA (LLOQ from Assay Validation Report #4648-00056, [redacted]

- 24 hours post-last injection: Low levels of viral DNA were detected in the urine (males only, <LLOQ), injection site (2 males and 3 females; 383-9,204 copies/µg gDNA), brain (males only, <LLOQ of 264 copies/µg gDNA), and duodenum (males only; 256 copies/µg gDNA).

- 4 weeks post-last injection: 1/10 brain samples were positive for viral DNA (male, <LLOQ); no other tissues evaluated were positive.

- 12 weeks post-last injection: 1/10 brain samples were positive for viral DNA (female; 2,427 copies/µg gDNA); no other tissues evaluated were positive.

Comment:

- Persistence of viral DNA levels in the brain potentially indicates relatively slow clearance from the brain.

Report Conclusion: OncoVEX^{GM-CSF} administered once weekly via s.c. injection to BALB/c mice for 12 weeks at dose levels of 1 x 10^5, 1 x 10^6 and 1 x 10^7 PFU/mouse/injection did not result in overt toxicity. Following weekly s.c. injections of OncoVEX^{GM-CSF} weekly to BALB/c mice for 5 weeks at 1 x 10^7 PFU/mouse/injection resulted in the presence of viral DNA in the urine, injection site, brain, and duodenum at

24 hours post-last injection, with clearance by 4 weeks with the exception of one brain sample that was positive at 4 weeks and one that was positive at 12 weeks post-last injection. There were no abnormal microscopic findings attributed to the product.

Comment:

- The expressed human GM-CSF is not bioactive in the mice, thus the findings are likely not related to exposure to this human protein.

5. **Document No.:** 4648-00029
   Study Report No.: 2024-060, conducted in compliance with GLP
   Report Date: June 1, 2009
   Title: OncoVEX^{GM-CSF} Repeat Dose Toxicity in the Mouse Followed by a 24 Hour or 4 Week Observation Period
   Objective: To compare the safety of repeat s.c. injection of OncoVEX^{GM-CSF} and OncoVEX$^{mouseGM-CSF}$ from manufacturing process [redacted]
   Testing Facility: [redacted].
   Test and Control Articles:
   - Test - OncoVEX^{GM-CSF} [redacted] Batch No. [redacted], purity 1x10^9 PFU/mg
     OncoVEX^{GM-CSF} [redacted] Batch No. [redacted], purity – 6x10^8 PFU/mg
     OncoVEX$^{mouseGM-CSF}$ [redacted] Batch No. [redacted], purity – 3.7x10^8 PFU/mg
     OncoVEX$^{mouseGM-CSF}$ [redacted] Batch No. [redacted], purity – 4.5x10^8 PFU/mg
   - Control - [redacted]

   Study Animals:
   - BALB/c mice (96/sex), 12-14 weeks old, 22.9-30.0 g for males, 17.4-23.1 g for females; purchased from [redacted]
   - Animals were assigned to groups using randomization by body weight.

   Study Design:
   - Study groups (6 mice/sex/sacrifice time point):
     - Group 1 - vehicle control, 6 mice/sex/sacrifice
     - Group 2 - OncoVEX^{GM-CSF} [redacted], 1x10^5 PFU/mouse/injection
     - Group 3 - OncoVEX^{GM-CSF} [redacted] 1x10^7 PFU/mouse/injection
     - Group 4 - OncoVEX^{GM-CSF} [redacted] 1x10^5 PFU/mouse/injection
     - Group 5 - OncoVEX^{GM-CSF} [redacted] 1x10^7 PFU/mouse/injection
     - Group 6 - OncoVEX^{mouseGM} [redacted] 1x10^7 PFU/mouse/injection
     - Group 7 - OncoVEX$^{mouseGM-CSF}$ [redacted] 1x10^5 PFU/mouse/injection
     - Group 8 - OncoVEX$^{mouseGM-CSF}$ [redacted] 1x10^7 PFU/mouse/injection

- Test and control articles were s.c. injected (100 µL/mouse/injection) on SDs 1, 4, 7, 10, and 13. The injection sites, located on the right and left hind quarter, were rotated for each injection. Animals were sacrificed at 24 hours (SD 14) and 28 days (SD 41) post-last injection.

- Evaluations:
    - Clinical observations -daily; physical examinations - weekly
    - Body weights - SDs -7 and 1, then weekly thereafter
    - Food consumption - weekly
    - Blood samples collected at sacrifice for clinical pathology (3 mice/sex/group each for hematology and clinical chemistry) and anti-HSV IgG analysis on the same animals
    - Urine samples were collected for 6-hour time periods on SDs 13/14 and 38/41 for urinalysis
    - Bone marrow smears were collected at sacrifice (not analyzed)
    - Comprehensive gross pathology (all animals) and histopathology for control (Group 1) and high dose groups (Groups 3, 5, 6 and 8)

Results:

- There were no unscheduled deaths.

- There were no abnormal clinical observations.

- There were no significant differences in mean body weight or mean food consumption values among the groups.

- For hematology parameters, as compared to the control group:
    - At 24 hours post-last injection (SD 14), 1/6 females each in Groups 6 and 8 had a 2-fold increase in reticulocyte counts; 1/6 Group 7 males had decreased reticulocyte counts.
    - At 28 days post-last injection (SD 41), 1/6 Group 5 males had increased reticulocyte counts.

- For serum chemistry parameters as compared to the control group:
    - On SD 14, 1/6 Group 7 males had increased potassium and creatinine levels; Groups 6-8 males had increased mean calcium levels.
    - On SD 41, 1/6 Group 8 females and males had increased AST and globulin levels; 1/6 males had increased creatinine levels.

- There were no differences in urinalysis parameters among the study groups.

- A large spleen was observed in Groups 5, 6, and 8 females on SD 14. On SD 41, no macroscopic findings attributed to the test articles were observed.

- Microscopic analysis at 24 hours post-last injection (SD 14):

- Cellulitis with inflammatory cell infiltration at both injection sites; the incidence and severity were dependent on the dose level and the number of injections. There were no significant differences between the incidence and severity of this finding between the [redacted] products.

Table 1. Incidences and severities of cellulitis at 24 hours post last injection (SD 14)

Group incidence of cellulitis: Injection sites – Interim kill

Tissue and finding	Level (pfu)	1M 0	2M $1x10^5$	3M $1x10^7$	4M $1x10^5$	5M $1x10^7$	6M $1x10^7$	7M $1x10^5$	8M $1x10^7$
		Males							
Injection site 1 cellulitis	No. examined:	6	1	6	0	6	6	1	6
	Grade -	6	0	0	0	0	0	0	1
	1	0	1	0	0	2	1	0	1
	2	0	0	4	0	2	1	1	2
	3	0	0	2	0	2	4	0	2
Injection site 2 cellulitis	No. examined:	6	0	6	0	6	6	0	6
	Grade -	6	0	1	0	1	2	0	0
	1	0	0	3	0	4	1	0	3
	2	0	0	2	0	1	3	0	3
	3	0	0	0	0	0	0	0	0

Level (pfu)	1F 0	2F $1x10^5$	3F $1x10^7$	4F $1x10^5$	5F $1x10^7$	6F $1x10^7$	7F $1x10^5$	8F $1x10^7$
	Females							
Injection site 1 cellulitis	No. examined:	6	1	6	1	6	6	1	6
	Grade -	6	0	0	0	1	1	0	0
	1	0	0	1	0	2	0	0	1
	2	0	1	5	1	2	2	1	2
	3	0	0	0	0	1	3	0	3
Injection site 2 cellulitis	No. examined:	6	0	6	2	6	6	1	6
	Grade -	6	0	2	0	1	0	0	0
	1	0	0	4	0	3	4	0	2
	2	0	0	0	2	2	2	1	3
	3	0	0	0	0	0	0	0	1

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate
Group 2 & 3: OncoVEX^{GM-CSF} (b) (4)
Group 4 & 5: OncoVEX^{GM-CSF}
Group 6: OncoVEX$^{mouseGM-CSF}$
Group 7 & 8: OncoVEX$^{mouseGM-CSF}$ [redacted]

- A slightly higher level of hematopoiesis was observed in the splenic red pulp of males from all test article groups; there were no significant differences in the incidence and severity of this finding between the [redacted] products. Lymphoid hyperplasia was also present in the splenic white pulp of both sexes injected with the high dose OncoVEX^{GM-CSF} [redacted] but not OncoVEX^{GM-CSF} [redacted] In contrast, lymphoid hyperplasia was present in both sexes injected with high dose OncoVEX$^{mouseGM-CSF}$ [redacted] products.

Table 2. Incidences and severities of hematopoiesis and lymphoid hyperplasia at 24 hours post last injection (SD 14)

Group incidence of selected microscopic findings: Spleen – Interim kill

Tissue and finding	Level (pfu)	1M 0	2M $1x10^5$	3M $1x10^7$	4M $1x10^5$	5M $1x10^7$	6M $1x10^7$	7M $1x10^5$	8M $1x10^7$
		Males							
Spleen	No. examined:	6	6	6	6	6	6	6	6
haemopoiesis	Grade -	0	0	0	0	0	0	0	0
	1	6	2	2	2	2	1	3	0
	2	0	4	4	4	4	5	3	5
	3	0	0	0	0	0	0	0	1
lymphoid hyperplasia	Grade -	6	6	6	6	4	2	6	2
	1	0	0	0	0	2	4	0	3
	2	0	0	0	0	0	0	0	1

	Level (pfu)	1F 0	2F $1x10^5$	3F $1x10^7$	4F $1x10^5$	5F $1x10^7$	6F $1x10^7$	7F $1x10^5$	8F $1x10^7$
		Females							
Spleen	No. examined:	6	6	6	6	6	6	6	6
haemopoiesis	Grade -	0	0	0	0	0	0	0	0
	1	3	1	2	1	1	2	1	0
	2	3	5	4	5	5	4	4	5
	3	0	0	0	0	0	0	1	1
lymphoid hyperplasia	Grade -	6	6	6	6	3	2	5	2
	1	0	0	0	0	0	2	1	3
	2	0	0	0	0	3	2	0	1

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate
Group 2 & 3: OncoVEX^{GM-CSF} (b) (4)
Group 4 & 5: OncoVEX^{GM-CSF}
Group 6: OncoVEX$^{mouseGM-CSF}$
Group 7 & 8: OncoVEX$^{mouseGM-CSF}$

- In the femoral and sternal marrow, increased myeloid hematopoiesis was observed in males receiving high dose OncoVEX^{GM-CSF} and OncoVEX$^{mouseGM-CSF}$ as compared to the controls, with no significant differences between animals dosed with [redacted] products. Increased myeloid hematopoiesis was displayed in the high dose females injected with OncoVEX^{GM-CSF} and OncoVEX$^{mouseGM-CSF}$ generated using [redacted] and in females injected with OncoVEX$^{mouseGM-CSF}$, as compared to the respective controls. However, no hematopoiesis was seen in females injected with OncoVEX^{GM-CSF} generated using [redacted]

Table 3. Incidences and severities of myeloid hematopoiesis at 24 hours post last injection (SD 14)

Group increased haemopoiesis - myeloid: Femur and sternal marrow – Interim kill

		Males							
		1M	2M	3M	4M	5M	6M	7M	8M
Tissue and finding	Level (pfu)	0	$1x10^5$	$1x10^7$	$1x10^5$	$1x10^7$	$1x10^7$	$1x10^5$	$1x10^7$
Femur + marrow	No. examined:	6	0	6	0	6	6	0	6
increased haemopoiesis – myeloid	Grade -	5	0	2	0	3	2	0	2
	1	1	0	4	0	3	4	0	4
Sternum + marrow	No. examined:	6	0	6	0	6	6	0	6
increased haemopoiesis – myeloid	Grade -	5	0	2	0	3	1	0	2
	1	1	0	4	0	3	5	0	4

		Females							
		1F	2F	3F	4F	5F	6F	7F	8F
	Level (pfu)	0	$1x10^5$	$1x10^7$	$1x10^5$	$1x10^7$	$1x10^7$	$1x10^5$	$1x10^7$
Femur + marrow	No. examined:	6	0	6	0	6	6	0	6
increased haemopoiesis – myeloid	Grade -	6	0	6	0	4	0	0	2
	1	0	0	0	0	2	6	0	4
Sternum + marrow	No. examined:	6	0	6	0	6	6	0	6
increased haemopoiesis – myeloid	Grade -	6	0	6	0	4	0	0	2
	1	0	0	0	0	2	6	0	4

Key: "-" = finding not present, 1 = minimal
Group 2 & 3: OncoVEX^{GM-CSF} (b) (4)
Group 4 & 5: OncoVEX^{GM-CSF}
Group 6: OncoVEX$^{mouseGM-CSF}$ (b) (4)
Group 7 & 8: OncoVEX$^{mouseGM-CSF}$

- Microscopic analysis at 28 days post-last injection (SD 41):
  - The findings at the injection site were resolving.
  - Minimal levels of lymphoid hyperplasia in the spleen were present in 1/6 males receiving high dose OncoVEX^{GM-CSF} generated using [redacted] and in 1/6 females receiving high dose OncoVEX$^{mouseGM-CSF}$ generated using [redacted]

Comment:

- Per the study report, the observed cellulitis and changes in myeloid hematopoiesis may be associated with focal dermatitis in the skin and subcutis.

Report Conclusion: Healthy mice (both genders) that were administered multiple s.c. injections of OncoVEX^{GM-CSF} or OncoVEX$^{mouseGM-CSF}$ that were manufactured using [redacted] did not display any significant differences in clinical signs, body weights, food consumption, clinical pathology, gross pathology or histopathology findings between the [redacted] products. The microscopic changes seen in the spleen and in the femoral and sternal marrow, as well as the injection site reaction observed on SD 14, resolved in a majority of the mice by SD 41.

Comment:

- The sponsor did not provide a rationale for the dosing schedule and the sacrifice time points. However, this reviewer considers these intervals acceptable based on the BD and clearance profiles following single s.c. administration in non-tumor

bearing mice (Document No. 4648-00030). The study data supported the dosing regimen of once every 2 weeks in the initial, early-phase clinical trial.

6. **Document No.:** 4648-00032
Study Report No.: 7241-100 XBV 001/024155, conducted in compliance with GLP
Report Date: January 11, 2011
Title: OncoVEX^{GM-CSF}: A Single Dose Toxicity Study in Male Dogs with Administration into the Prostate
Objective: To evaluate toxicity and BD following bolus administration of OncoVEX^{GM-CSF} into the prostate of dogs
Testing Facility:
Test and Control Articles:
   - Test - OncoVEX^{GM-CSF}, Lot No.
   - Control -

Study Animals: A total of 6 male pure-bred beagle dogs (65-71 weeks old, 6.5-8.5 kg) were purchased from . the animals were inoculated against distemper, adenovirus type 2, parainfluenza, parvovirus, leptospirosis, rabies, oral papilloma, canine adenovirus type 2, para-influenza, and Bordetella.

Study Design:

- The dogs (2 /group) were administered OncoVEX^{GM-CSF} via intraprostatic injection into both lobes of the prostate under general anesthesia at dose levels of $3.57x10^4$ PFU/lobe or $2.5x10^6$ PFU/lobe. Two uninjected dogs were served as controls. Animals were sacrificed on day 14.

- Evaluations:
    - Animals were observed for 2 weeks post-injection
    - Clinical observations (daily), food consumption (daily), body weights (weekly)
    - Blood sample collection once at baseline and on days 2, 7, and 13 for hematology and clinical chemistry
    - Urine sample collection at baseline and during week 2
    - Complete gross pathology was conducted
    - Histopathology on selected tissues (testes, epididymides, prostate/ injection site, spinal cord, brain spleen, urinary bladder, and penile urethra)

- BD assessment:
    - Blood sample collection at baseline; 1, 4, 8, 24 and 48 hours; and 7 and 14 days post-injection
    - Urine sample collection at baseline; 24 and 48 hours; and 7 and 14 days post-injection
    - Tissues - testes, epididymides, cervical and lumbar spinal cord, brain, urethra, injection site and innervating nerve to the prostate
    - The qPCR assay was validated, with an assay sensitivity of ≥64 copies/μg gDNA

Results:

- There were no unscheduled deaths and no significant differences were observed among the study groups for clinical observations, body weights, food consumption, hematology, clinical chemistry, urinalysis, gross pathology, or histopathology.

- qPCR analysis ($3.57x10^4$ PFU/lobe):
  - Viral DNA was detected in the right epididymis (149.6 copies/µg gDNA) and at the injection site (right lobe; 68.1 copies/µg gDNA) of 1/2 animals.
  - No viral DNA was detected in blood or in any other collected tissues.

- qPCR analysis ($2.5x10^6$ PFU/lobe):
  - Viral DNA was detected in the right epididymis (<64 copies/µg gDNA), cervical spinal cord (4516.2 copies/µg gDNA) and lumbar spinal cord (657.8 copies/µg gDNA) of 1/2 animals.
  - Viral DNA was also detected in a blood sample collected at 8 hours post-injection from the other animal (1068.9 copies/µg gDNA).
  - No viral DNA was detected in any other blood or collected tissue samples.

Report Conclusion: A single intraprostatic injection of OncoVEX^{GM-CSF} in dogs resulted in transient localization of viral DNA in the injected tissue at both dose levels. Transient systemic distribution of viral DNA (blood) occurred with injection of $2.5x10^6$ PFU/lobe. No adverse effects were observed in any animal.

Comment:

- The sponsor administered HSV virus expressing huGM-CSF. No data were provided confirming bioactivity the amino acid homology of canine GM-CSF to huGM-CSF, or the permissiveness of the dog to the administered HSV virus. Thus, the biological relevancy of this animal species and route of administration is unknown.

Toxicology Studies Designed to Evaluate Mechanism of Toxicity:

7. **Document No.:** 4648-00024; non-GLP
   Report Date: November 05, 2010
   Title: The Effect of Acyclovir on the Replication of OncoVEX^{GM-CSF}
   Objective: To establish the relative sensitivity of OncoVEX^{GM-CSF} and the parental wt HSV-1 strain JS1, to acyclovir.
   Testing Facility: BioVex Ltd.
   Test Articles: OncoVEX^{GM-CSF}, Batch No. [redacted]

   Methods: The [redacted] consisted of [redacted]

(b) (4)

Results:

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Report Conclusion: The results indicate that OncoVEX^{GM-CSF} is susceptible to a similar concentration of acyclovir.

Comment:

- The IC_{50} value of acyclovir on OncoVEX^{GM-CSF} (0.23 – 0.39 mg/μL) appears to be comparable to the value on JS1 (0.22 -0.25 mg/mL). This indicates that the current clinical dose of acyclovir would be effective on the product.

8. **Document No.:** 4648-00052; non-GLP
Title: Assessment of the Effect of Repeat-Dose Subcutaneous Administration of OncoVEX$^{mouseGM-CSF}$ Viruses in Female BALB/c Mice
Objective: To evaluate the safety of repeated s.c. injections of OncoVEX$^{mouseGM-CSF}$ in female BALB/c mice and to assess the humoral response to the virus.
Testing Facility: BioVex Ltd.
Test and Control Articles:
    - Test - OncoVEX$^{mouseGM-CSF}$ Batch No.
    - Control -
Study Animals:
    - A total of 20 female BALB/c mice (4-6 weeks old, 16-20 g) were purchased from

Study Design:

- The mice (5 mice/group) were s.c. injected with control or test articles (100 μL/mouse/injection) on SDs 1, 4, 7, 11, and 14.
    - Group 1 - Vehicle
    - Group 2 - OncoVEX$^{mouseGM-CSF}$, 10^5 PFU/mouse/injection
    - Group 3 - OncoVEX$^{mouseGM-CSF}$, 10^6 PFU/mouse/injection
    - Group 4 - OncoVEX$^{mouseGM-CSF}$, 10^7 PFU/mouse/injection

- Evaluations consisted of clinical observations (daily up to SD 46), body weights (SD 1 and every 3-6 days thereafter), and collection of blood samples at sacrifice (SD 46) for measurement of anti-HSV antibodies.

Comment:

- Although a humoral response to rhuGM-CSF in humans has been well documented, no measurement of antibody levels against muGM-CSF was performed in the mice.

Results:

- No abnormal clinical signs were observed and there were no differences in mean body weights among the study groups.
- Anti-HSV antibody titers were detected in all Groups 2-4 mice; levels increased in a dose-dependent manner (Table 1).

Table 1. Serum Anti-HSV Antibody Levels at SD 46

Virus Dose PFU/animal	Antibody Units	Average Antibody Units
Control	<1, <1, <1, <1, <1	<1
1×10^5	37, 40, 20, 29, 14	28
1×10^6	50, 60, 48, 48, 52	51.6
1×10^7	38, >100, >100, >100[a]	>100

Report Conclusion: Repeat administration of OncoVEX$^{mouseGM\text{-}CSF}$ up to 10^7 PFU/mouse did not result in adverse findings. Anti-HSV-1 antibody titers were detected in the low and high dose groups.

9. Document No.: 4648-00014; non-GLP
Title: Assessment of Neurovirulence Following Intranasal Administration of OncoVEX$^{GM\text{-}CSF}$ in Female BALB/c Mice
Objective: To evaluate the potential for neurovirulence following intranasal administration in female BALB/c mice.
Testing Facility: BioVex Ltd.
Test Article: OncoVEX$^{GM\text{-}CSF}$, Batch No. [redacted]

Methods: A total of 30 female BALB/c mice (age, weight, supplier not provided) were randomized into 3 groups (10 mice/group). Mice were administered OncoVEX$^{GM\text{-}CSF}$ at 1×10^4, 10^5, or 10^6 PFU/mouse via intranasal instillation (20 µL/mouse) in a drop-wise manner under [redacted] anesthesia**.** Mice were observed daily for 24 days post-dose.

Results: None of the animals displayed signs of ill health.

Report Conclusion: The results indicate that mice tolerated the intranasal administration of OncoVEX$^{GM\text{-}CSF}$ up to 10^6 PFU/mouse.

Comment:

- OncoVEX^{GM-CSF} expresses human GM-CSF, which is not active in mice, thus the interpretability of these data are questionable.

10. Document No.: 4648-00004; non-GLP

Title: Assessment of Effect of Intra Cerebral Administration of OncoVEX Viruses in Female BALB/c Mice

Objective: To evaluate the safety of intracerebral administration of OncoVEX viruses in BALB/c mice

Testing Facility: BioVex Ltd.

Test and Control Articles:

- Test –

Test Articles

Virus	
17*syn*+/34.5-/	
JS1/34.5-/CMV	
OncoVEX$^{mouseGM-CSF}$	

- Control -

Study Animals:

- A total of 50 female BALB/c mice (age and weights not provided) were purchased from

Study Design:

- Mice assigned to the groups depicted in Table 1, Mice were injected via the intracerebral (i.c.) route (20 μL/mouse) according to the design specified in Table 1 below. The animals were observed every 3 days until sacrifice (35 days post-dose); body weights were recorded every 4 days.

- Blood samples were collected at sacrifice for antibody analysis by

Table 1. Study Groups

Group	N	Virus	Virus Concentration (PFU/mL)	Virus Dose (PFU/animal)
1	5	Control	-	-
2	5	17*syn*+/34.5-/[redacted]	$5x10^4$	$1x10^3$
3	5	17*syn*+/34.5-/CMV[redacted]	$5x10^5$	$1x10^4$
4	5	17*syn*+/34.5-/CMV[redacted]	$5x10^6$	$1x10^5$
5	5	JS1/34.5-/CMV[redacted]	$5x10^4$	$1x10^3$
6	5	JS1/34.5-/CMV[redacted]	$5x10^5$	$1x10^4$
7	5	JS1/34.5-/CMV[redacted]	$5x10^6$	$1x10^5$
8	5	OncoVEX$^{mouseGM-CSF}$	$5x10^4$	$1x10^3$
9	5	OncoVEX$^{mouseGM-CSF}$	$5x10^5$	$1x10^4$
10	5	OncoVEX$^{mouseGM-CSF}$	$5x10^6$	$1x10^5$

Results:

- No adverse clinical signs were displayed in Groups 2-4 mice.
- No adverse clinical signs were observed in 13/15 animals in Groups 5-7; however, 1/5 animals each in Groups 5 and 6 had ear infections at approximately one week post-dose, and were sacrificed.
- No adverse clinical signs were observed in 12/15 animals in Groups 8-10. However, 1/5 Group 10 mice was found dead on the day after dosing, 1/5 died two days after dosing, and 1/5 displayed abnormal clinical signs at 7 days post-injection and was subsequently sacrificed.

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- Mean body weights were similar among the groups throughout the study.
- Antibody titers (Ab units = 5-40) against HSV-1 were detected in Groups 2-10.

Report Conclusion: Per the sponsor, previously published work by MacLean et al., cites an LD_{50} of <10 PFU when wt HSV (strain 17*syn*+) is administered to mice by the i.c. route. The results from the above study indicate that JS1/34.5- and OncoVEX$^{mouseGM-CSF}$ are therefore significantly attenuated following i.c. administration as compared to the wt strain 17*syn*+.

Reference:

1. MacLean AR. HSV-1 type deletion variants 1714 and 1716 pinpoint neurovirulence-related sequences in Glasgow strain 17 *syn+* between intermediate early gene 1 and the 'a' sequence. J Gen Virol 729 pt3):631-639, 1991

11. Document No.: 4648-00015; non-GLP
Report Date: December 10, 2010
Title: A Comparative Assessment of 17*syn+* and OncoVEX Virus Reactivation from Latency in the Peripheral Nervous System of BALB/c Mice
Objective: To evaluate the reactivation capabilities of OncoVEX^{GM-CSF} compared with wild type strains 17*syn+* and JS1, following footpad administration of virus and explant of dorsal root ganglia (DRGs).
Testing Facility: BioVex Ltd.
Test Articles:

- Test –
  - OncoVEX^{GM-CSF}, Batch No. [redacted]
  - wt HSV-1 clinical isolate JS1
  - Laboratory strain 17*syn+*

Study Animals:

- A total of 36 female BALB/c mice (4-6 weeks old, 16-20 g) were purchased from [redacted].

Study Design:

- Mice were randomized to three groups (12 mice/group); $2x10^4$ PFU/mouse (20 µL/mouse) of each virus was injected into the rear foot pad. The animals were monitored for any signs of peripheral nervous system (PNS) infection.

- At 3 days post-injection (acute infection phase), 2 mice/group were sacrificed, and DRG L1-6 (which innervate the injection site) were isolated and prepared for testing infectivity in BHK cells.

- The remaining mice were monitored for an additional 21-23 days to allow for establishment of a latent infection. Mice were sacrificed at 24 (JS1), 25 (OncoVEX^{GM-CSF}), and 26 (17 *syn+)* days post-injection and [redacted] The samples were then [redacted]

Results:

- Two animals in the JS1 group developed hind leg paralysis in both legs: one animal was sacrificed on day 8 and the other on day 10. The third animal in this group displayed restricted movement in one hind leg and was noted to be limping beginning on day 12. This animal was monitored throughout the study and did not develop additional findings.

- None of the animals in the OncoVEX^{GM-CSF} and 17s*yn*+ groups showed any signs of PNS infection during the study.

- In the acute infection phase, no virus was recovered from DRG samples from any animal, indicating that virus replication was not occurring at this time point.

- In the latent infection phase latent infections with all three viruses (JS1 and 17*syn*+ and OncoVEX^{GM-CSF}) were established in the lumbar DRGs, which could be reactivated *in vitro*. Significantly higher levels of reactivation were observed in the JS1 and OncoVEX^{GM-CSF} groups (5/8 and 9/10 animals, respectively) compared to the 17*syn*+ group (1/10 animals).

Report Conclusion: The results show that all three strains of HSV tested, JS1 and 17*syn*+ and OncoVEX^{GM-CSF}, established latent infections in murine DRGs following injection into the footpad.

References:

1. Whitby AJ, Blyth WA, and Hill TJ. The effect of DNA hypomethylating agents on the reactivation of herpes simplex virus from latently infected mouse ganglia in vitro. Brief report. Arch Virol. 97:137-144, 1987.
2. Bernstein DI and Kappes JC. Enhanced in vitro reactivation of latent herpes simplex virus from neural and peripheral tissues with hexamethylenebisacetamide. Arch Virol. 99:57-65, 1988.
3. Smith J, Thomas SK, Coffin RS, and Latchman DS. Examination of the potential interactions between herpes simplex viruses and replication-competent virus in vitro and in vivo. Gene Ther Reg. Vol2, N1:29-47, 2003.

Developmental and Reproductive Toxicology Studies:

12. Document No.: 117250
Study Report No.: 20035379; conducted in compliance with GLP
Report Date: April 24, 2013
Title: Talimogene Laherparepvec: Intravenous Embryo-Fetal Development Study in the BALB/c [redacted] Mouse
Objective: To evaluate the potential developmental and reproductive toxicity of Talimogene Laherparepvec (OncoVEX^{GM-CSF}).
Testing Facility: [redacted]
Test and Control Articles:
Test - OncoVEX^{GM-CSF}, Batch No. [redacted], titer 10^8 PFU/mL
Control - Vehicle [redacted] sodium chloride, [redacted] sorbitol and [redacted] myo-inositol, [redacted]

Study Animals:

- A total of 197 non-pregnant female BALB/c [redacted] mice, 61 days old, 18.3-21.7 g were purchased from [redacted]. Male mice (same strain and supplier) were provided by the Testing Facility.
- Two female mice were mated with one male mouse for a maximum of 5 days; mice were examined daily for a copulatory plug *in situ*.
- Female mice with a copulatory plug (considered gestational day (GD) 0) were randomized to groups based on a computer-generated, weight-ordered randomization procedure and housed individually.

Study Design:

- Main Study (35 females /group) and Toxicokinetic (TK) Study (6 females/group) animals:
  - Group 1 - vehicle control
  - Group 2 - OncoVEX^{GM-CSF} 1x10^5 PFU/mouse/injection
  - Group 3 - OncoVEX^{GM-CSF} 1x10^6 PFU/mouse/injection
  - Group 4 - OncoVEX^{GM-CSF} 1x10^7 PFU/mouse/injection

- Mice were i.v. injected (0.1 mL/mouse/injection) with the test and control articles on GDs 6, 9, 12, and 15.

Comments:

- The route of administration is not the intended clinical route; the sponsor did not provide a rationale for use of this route or dosing schedule.

- This study cannot be used to evaluate potential reproductive toxicity of huGM-CSF because huGM-CSF encoded by OncoVEX^{GM-CSF} is not active in mice.

Table 1. Study Design

Group No.	Test Material	Dose Level (PFU/mouse)	Concentration (nominal - PFU/mL)	Dose Volume (mL/mouse)	Main Study Mouse Numbers	Toxicokinetic Mouse Numbers
1	control article	0	0	0.1	4301 - 4335	4441 - 4446
2	talimogene laherparepvec	1 x 10^5	1 x 10^6	0.1	4336 - 4370	4447 - 4452
3	talimogene laherparepvec	1 x 10^6	1 x 10^7	0.1	4371 - 4405	4453 - 4458
4	talimogene laherparepvec	1 x 10^7	1 x 10^8	0.1	4406 - 4440	4459 - 4464

PFU = Plaque Forming Units.

- In-life maternal evaluations:
  - Mortality and moribundity - twice daily; clinical observations - daily; physical examination - weekly
  - Body weights at pre-mating, GD 0, daily thereafter beginning on GD 6

- Food consumption (Main study only) - twice pre-mating and on GDs 0, 6, 9, 12, 15, and 18

- Maternal and fetal blood sample collection (TK study):
  - Dams were sacrificed on GD 18 and blood samples collected via the vena cava
  - Fetal blood samples were collected on GD 18 from all viable fetuses in each litter; samples were pooled for each litter
- Maternal terminal evaluations:
  - For all unscheduled deaths - pregnancy status (implantation sites and number of corpora lutea), gross pathology, and tissue collection
  - Main study - sacrifice on GD 18 pregnancy status and uterine contents (implantation sites and number of corpora lutea)
  - Macroscopic pathology - placenta (size, color and shape), live and dead fetuses, and late resorptions
  - The uterus of all apparently non-pregnant animals was examined to confirm the absence of implantation sites
  - Gross pathology was conducted on tissues of the thoracic, abdominal, and pelvic cavities, and a comprehensive list of tissues was harvested and archived for possible histopathology evaluation

- Fetal terminal evaluations:
  - Examined for sex identification and external abnormalities; late resorptions and dead fetuses were examined similarly to the extent possible
  - Approximately half of the fetuses in each litter was examined for visceral abnormalities [redacted] The remaining half was examined for skeletal abnormalities after staining [redacted]

Results:

- There were three unscheduled maternal deaths; none were related to administration of OncoVEX^{GM-CSF}: one dam (not pregnant) was injured during attempts to recapture the animal after it escaped; the second (not pregnant) and third (pregnant) dam died immediately following the i.v. injection.

- There were no clinical signs attributed to OncoVEX^{GM-CSF} displayed by the dams.

- No significant differences in mean body weight gains were observed for dams in Groups 1-3. Following the second injection of OncoVEX^{GM-CSF} significantly decreased mean body weights were displayed by Group 4 on GDs 6-7 and GDs 9-10 compared to concurrent controls. This finding was not observed for the remainder of the gestation interval. Gravid uterine weights were not significantly different among the study groups.

- Mean food consumption at GDs 6-15 for dams in Groups 2-4 was slightly decreased compared to the controls (86%, 83% and 87%, respectively). This finding was not dose-dependent.

- There were no test article-related macroscopic findings; ovarian and uterine examinations showed the following:
    - Pregnancy was confirmed in 21/35, 15/35, 18/35, and 15/35 mice in Groups 1-4 4, respectively.
    - There were no significant differences between groups in the mean number of corpora lutea and implantations in each litter. The mean percent preimplantation and post-implantation loss, resorbed conceptuses, live male fetuses were similar across study groups. The mean litter size, live fetuses, early and late resorptions, and fetal body weights were similar between groups.
    - No dam had a litter consisting of only resorbed conceptuses and all placentae appeared normal.
    - There was a significant difference in the fetal sex ratio in the Group 3 litters as compared to the other groups.

- No abnormalities were observed in fetal external or soft tissue, and no skeletal or fetal ossification abnormalities were found.
    - Fetal evaluations were based on 147, 118, 117, and 121 live fetuses from 21, 15, 18, and 15 litters, respectively, in Groups 1-4, respectively.
    - Open eyelids occurred in one fetus each in Groups 1 and 2.
    - A variation in vessel development occurred across the four groups at a similar incidence. An extra site of ossification between the frontal bones and the presence of a cervical rib at the 7^{th} cervical vertebra were also observed at a similar incidence across all groups.
    - One Group 4 fetus had a skeletal malformation (fused arches) and other skeletal variations.

- qPCR analysis (TK study):
    - A total of 6 dams/groups were dosed. Pregnancy was confirmed in 5/6, 2/6, 3/6, and 4/6 mice in Groups 1-4, respectively.
    - The LLOQ was ≥46 copies/µg gDNA (Study No. LR-BID-0019); all blood samples were negative for qPCR inhibitors.
    - Dams - Viral DNA was detected in the blood of all Groups 2-4 dams. The mean levels reflected a dose-dependent increase (approximately 7.2 $x10^4$, 6.5 $x10^5$, and 4.7 $x10^6$ copies/µg gDNA in Groups 2-4, respectively).
    - Fetuses - Mean viral DNA in pooled blood samples (per litter) was below the LLOQ for Groups 2 and 3. One pooled blood sample (i.e., one litter) from Group 4 had a very low DNA levels (approximately 44 copies/µg gDNA).

Comments:

- The report stated that the amount of DNA detected in the fetal blood sample was <0.001% of the viral DNA level in the blood of the animal. However, this estimation does not account for the differences in blood volume of the mother and of individual dams, thus does not represent the relative exposure level to the fetus.

- The sponsor concluded that repeat i.v. injection of OncoVEX^{GM-CSF} at dose levels up to 1x10^{8} PFU/mouse/injection in pregnant mice did not result in adverse maternal or embryo-fetal findings. However, this conclusion excludes potential risk associated with human GM-CSF because this human protein is not biologically active in mice.

Other Toxicology Studies:

13. Document No.: 118737; non-GLP

Title: Talimogene laherparepvec (AMG 678, OncoVEX^{GM-CSF}): Tolerability and Anti-Tumor Effects on Human Colorectal Carcinoma (HT-29) Tumors in [redacted] SCID mice and BALB/c nude mice

Objective: To evaluate the tolerability and anti-tumor activity of talimogene laherparepvec following repeated i.t. injection in HT-29 colorectal carcinoma tumors implanted into [redacted] SCID mice and BALB/c nude mice.

Testing Facility: Amgen Inc.

Test and Control Articles:

- Test - OncoVEX^{GM-CSF}, Batch No. [redacted] PFU/mL
- Control - Vehicle, Batch No [redacted]

Study Animals:

- Thirty female [redacted] SCID mice and 18 female BALB/c nude mice (11 weeks old) were purchased from [redacted]

Study Design:

- On SD -8, 5x10^{6} HT-29 human colorectal carcinoma cells (passage 3, supplied by Amgen Oncology Cell Bank) were s.c. injected (100 µL) into the right flank of each mouse. The tumor volume, determined as length and width [(W^2xL)/2], was measured twice weekly. When tumor volumes reached approximately 100 mm^{3}, the mice were randomly assigned to study groups using a randomization spreadsheet (not provided) generated by the testing facility.

- Per the report, since tumors implanted in BALB/c nude mice exhibit a slower growth rate relative to SCID mice, dosing initiated on SD 1 for the SCID mice and on SD 4 for the BALB/c mice. SCID mice were i.t. injected with vehicle (50 µL/mouse/injection) or OncoVEX^{GM-CSF} at 5x10^{6} PFU/mouse/injection on SDs 1, 4, and 7. BALB/c mice were i.t. injected at the same virus dose level and volume on SDs 4, 7, and 10:

  - Group 1 - [redacted] SCID, 10 mice - Uninjected
  - Group 2 - [redacted] SCID, 10 mice - Vehicle
  - Group 3 - BALB/c nude, 9 mice - Vehicle
  - Group 4 - [redacted] SCID, 10 mice - OncoVEX^{GM-CSF}
  - Group 5 - BALB/c nude, 9 mice - OncoVEX^{GM-CSF}

- Body weights - SDs -1, 17, and 21 for all mice; SD 18 for [redacted] SCID mice, and SDs 23, 25, 28, and 32 for BALB/c nude mice

Results:

- Intratumoral injection of OncoVEX^{GM-CSF} resulted in regression of HT-29 tumors in [redacted] SCID mice, with mean tumor volumes reduced by 69 ±20% (range = 31-96%). BALB/c nude mice exhibited reduction in mean tumor volume of 80 ±9% (range = 71-100%) compared to the SCID mice.

- Unscheduled sacrifices (due to weight loss, hypoactivity, and/or poor response to stimuli):
  - On SDs 18-21, six Group 4 mice - select tissues were collected from 3/6 mice
  - On SD 21 - four Group 2 and four Group 4 mice - a comprehensive list of tissues was collected

- Abnormal clinical signs were observed only in the Group 4 mice, and included ruffled fur (3/10 mice on SD 17) and hypoactivity and poor response to stimuli (4/10 mice starting on SD 18).

- Body weights: Groups 3 and 5 had a ~4% body weight gain over the 32-day study period relative to SD 1. Group 4 mice displayed a mean decrease of 6 ±4% (n = 10, range 0-12%) on SD 17 relative to SD 1. Group 4 mice that survived to SD 21 exhibited a mean body weight decrease of 15 ±2% compared to SD 1.

- The Group 4 mice had flaccid and distended distal small and large intestines with fluid (10/10) and ulcerated skin overlying the tumor site (4/10).

- Histopathology findings in the Group 4 mice were related to OncoVEX^{GM-CSF} administration:
  - Gastrointestinal (GI) tract: minimal to marked myenteric neuron necrosis; presence of intranuclear inclusion bodies in the jejunum (4/6 mice), ileum (5/6 mice), cecum (4/6 mice), proximal colon (4/6 mice) and distal colon (4/6 mice)
  - Skin: moderate to marked necrosis with intranuclear inclusion bodies overlying the tumor (4/5 mice); mild skin ulcers in the dorsal skin (3/5 mice) and perineal skin (2/3 mice)

- Brain: mild, focal neuronal intranuclear inclusion bodies in brainstem neurons (1/7 mice); moderate necrosis and intranuclear inclusion bodies in the pineal gland (1/7 mice)
- Adrenal glands: mild to marked foci of necrosis with intranuclear inclusion bodies in the adrenal cortex (5/5 mice)
- Pancreas: minimal to mild foci of necrosis with intranuclear inclusion bodies in the pancreatic [redacted] (2/5 mice)
- Eye: minimal to moderate foci of retinal necrosis with intranuclear inclusion bodies and mild to moderate endophthalmitis in the eye (2/4 mice)

Report Conclusion: The BALB/c nude mice were not adversely affected following i.t. injection of OncoVEX^{GM-CSF}. However, the [redacted] SCID mice exhibited weight loss and abnormal clinical signs resulting in unscheduled sacrifices. The presence of viral inclusion bodies in the brain indicates viral replication consistent with HSV-1 infection. Histopathology of tissues from the [redacted] SCID mice showed adverse findings in multiple organs, including enteric neurons of the GI tract, which may have contributed to the impaired GI function and weight loss.

Comments:

- The adverse findings obtained in SCID mice receiving OncoVEX^{GM-CSF} indicate HSV-1 infectivity under a worst case scenario (immunosuppression). SCID mice are more sensitive to HSV-1 due to severe immune suppression as compared to nude mice.

- Human GM-CSF is not biologically active in mice, thus any observed adverse effects would be caused by the virus alone.

14. Study No.: S13M-01304; non-GLP
Report Date: November 15, 2013
Title: Talimogene laherparepvec: Pathology Report for, "T-VEC efficacy in [redacted]
Objective: This report summarizes gross pathology and histopathology results obtained from analyses of a panel of tissues harvested from three female BALB/c nude mice in a pharmacology study (Study #20130916, not submitted in the BLA) that were sacrificed early.

Comment:

- The study report for Study #20130916 was not submitted in the BLA; however, the summary information provided is adequate.

Testing Facility: Amgen Inc.
Test and Control Articles:

- Test - OncoVEX^{GM-CSF}, Batch No. [redacted]
- Control – Vehicle [redacted]

Methods:

- Forty female BALB/c nude mice (10 mice/group) were s.c. injected with [redacted] sarcoma cells into the left flank. Mice were i.t. injected with vehicle (Group 23- 50 µL/mouse/injection) or OncoVEX^{GM-CSF} at $5x10^4$ to $x10^6$ PFU/mouse/injection (Groups 1-2 and 4) on SDs 1, 4, and 7. Gross pathology and histopathology analyses were conducted on a panel of tissues collected from one mouse each in Groups 2, 3 and 4 that were sacrificed early.

Results:

- The Groups 2 and 4 mice had thin, flaccid, and fluid-distended intestines (the ileum, cecum, and large bowel). A large ulcerated area of skin overlying the tumor was also present in the Group 2 mouse.

- Microscopic findings attributed to injection of OncoVEX^{GM-CSF} were identified in the following tissues/organs:
    - GI tract: myenteric neuron necrosis and/or intranuclear inclusion bodies in the proximal and distal colon (mild to severe) with mural smooth muscle necrosis (mild to moderate). The presence of intranuclear inclusion bodies and necrosis of myenteric neurons in the colon likely led to impaired peristalsis, thin flaccid/fluid filled intestines, and weight loss – both mice
    - Skin and tumor: moderate epithelial necrosis with intranuclear inclusion bodies in the tumor and ulcerated skin overlying the tumor of the Group 2 mouse; correlating with the skin lesion observed
    - Adrenal gland: mild necrosis and intranuclear inclusion bodies (Group 4 mouse)

Report Conclusion: The presence of viral inclusion bodies in multiple tissues (GI tract, tumor, and adrenal gland) indicates active viral replication and infection in these tissues. Microscopic changes in the GI tract may have caused the impaired GI function and weight loss.

Comments:

- The sacrifice time points and the study duration were not provided.

- The objective of this study was also addressed in Document No. 118737, which was a better-designed study that included both SCID and BALB/c mice.

15. Study No.: 4648-00031
Study Report No.: 11964.01.02; conducted in compliance with GLP
Report Date: September 02, 2007
Title: GLP Toxicity Study of HSV vector OncoVEX^{GM-CSF} Administered by the Intrahepatic Arterial Route to Rats
Objective: To determine the toxicity of OncoVEX^{GM-CSF} when administered via the intrahepatic artery in rats
Testing Facility: [redacted]

Test and Control Articles:

- Test - OncoVEX^{GM-CSF}, Batch No. [redacted], titer 10^7 PFU/mL
- Control - Vehicle [redacted]

Methods:

[redacted] (6 rats/sex/group) were administered dosed with vehicle or OncoVEX (1 x 10^5 or 1 x 10^7 PFU/rat) via the intrahepatic route on SD 1.Clinical observations were performed twice daily on SDs 1-7 and 8, 15, 21, and 29. Body weight and food consumption were measured weekly. On SDs 5 and 29, blood was collected for hematology and chemistry from 3 rats/sex/group/time point, followed by sacrifice. Complete gross pathology and histopathology were performed on a comprehensive list of tissues.

Results:

There were no unscheduled deaths, and no abnormal test article-related changes for any parameter evaluated. Various inflammatory and reactive changes noted in the liver and adjacent abdominal organs on SD 5 were similar in incidence and severity between control and test groups (due to the surgical and injection procedures). By SD 29, these findings were resolving.

Report Conclusion: Administration of OncoVEX^{GM-CSF} up to 10^7 PFU/rat into the hepatic artery was well tolerated in rats.

Comment:

- The route of administration did not reflect the clinical route, and a rationale was not provided.

CONCLUSION

The pharmacology studies suggest that T-VEC (expressing human GM-CSF) is associated with anti-tumor activity. The *in vitro* studies showed that T-VEC is cytotoxic when exposed to murine and human cells of various solid tumor types. Bioactivity was confirmed with *in vivo* studies conducted in syngeneic tumor-bearing mice administered T-VEC or OncoVEX$^{murineGM-CSF}$. A relatively lower anti-tumor response was displayed in syngeneic tumor-bearing mice administered T-VEC compared to mice injected with OncoVEX$^{murineGM-CSF}$. Anti-tumor activity was also observed in noninjected tumors that were distant to the tumor that was injected with OncoVEX$^{murineGM-CSF}$; however, this effect was notably reduced compared to that for the injected tumor. Mice displayed protection against tumor re-challenge up to 6 months following i.t. injection of T-VEC. A T-cell mediated immune response, measured by IFN-γ release, was documented in a syngeneic murine reticulum cell sarcoma (A20) model following i.t. administration of OncoVEX$^{murineGM-CSF}$ or the OncoVEX backbone. Measurable levels of hGM-CSF were detected in the tumors, with very low levels in the blood, following i.t. injection of T-VEC to A20 tumor-bearing mice. Immune competent mice pre-immunized to wt HSV-1 exhibited an anti-tumor response to T-VEC that was similar to that in mice that were not

pre-immunized, indicating that the presence of pre-existing [redacted] did not negatively impact anti-tumor activity. Tumor-bearing mice that were administered an immunosuppressive agent [redacted] also showed anti-tumor activity following administration of T-VEC.

The T-VEC biodistribution and shedding profiles were evaluated using a qPCR assay that quantified viral DNA levels. The results showed that following i.t. injection in mice bearing murine B cell lymphoma, viral DNA was predominantly present in the tumor, blood, and tissues likely associated with immune-mediated viral clearance (e.g., spleen). Low levels of viral DNA were detected in the brain and in highly perfused tissues; however, this was not associated with abnormal histopathology findings.

Following s.c. injection of T-VEC in healthy immunocompetent BALB/c mice, viral DNA levels remained primarily at the injection site and clearance was nearly complete by 28 days post-injection. After i.v. injection the virus was present predominantly at the injection site and in the blood. Low levels of viral DNA were detected in the blood, liver, spleen, trigeminal ganglia, and heart up to 56 days post-injection. Low levels of viral DNA were also detected in the urine following s.c. administration up to 24 hours post-injection and no DNA was detected in the urine following i.v. injection. However, because the qPCR assay only detects a fragment of viral DNA, it does not inform as to whether the virus is intact and capable of causing infection.

Toxicology studies were conducted to evaluate the safety of T-VEC. Although human GM-CSF transcript levels were detected in mice, the protein is not biologically active in this species. However, T-VEC displays anti-tumor activity in mice. Thus, potential T-VEC-related adverse findings observed in the mice were likely due to the HSV-1 component. Following single s.c. administration in nontumor-bearing immunocompetent BALB/c mice, a no-observed-adverse-effect-level (NOAEL) of 10^7 PFU/kg was achieved, which is approximately 1.7-fold higher than the maximum clinical dose level specified in the proposed product label ($4x10^8$ PFU/subject [$5.7x10^6$ PFU/kg]). Repeat s.c. administration of T-VEC in healthy mice resulted in transient inflammation at the injection site, clinical pathology and histopathology findings in the spleen, bone marrow, and lymphoid tissues. These findings reflect the development of anti-viral immunity. Studies evaluating potential neuropathologic or neurovirulence effects of OncoVEX$^{mouseGM-CSF}$ in immunocompetent mice showed no adverse findings following intracranial administration of 10^4 PFU/mouse.

Immunodeficient mice, including SCID (deficient in T and B cells) and BALB/c nude strains (deficient in T cells), bearing H-29 tumors were i.t. injected with T-VEC. Viral infection was systemic and accompanied by adverse findings in various non-tumor tissues (e.g., gastrointestinal tract, brain) and body weight loss. These findings were consistent with those reported in immunocompetent or immunodeficient mice following wt HSV-1 infection. However, the incidence of lethality was lower in the nude mice and the time to death was delayed for the SCID mice, as compared to the respective mice injected with wt HSV-1. Plaque reduction assay data indicate that T-VEC is sensitive to acyclovir, potentially supporting its use to mitigate adverse effects related to HSV infection following administration of T-VEC.

Immunocompetent mice administered OncoVEX$^{murineGM-CSF}$ via the i.c. route survived longer compared to published data for mice injected with wt HSV-1 alone via the same route. However, following administration of T-VEC into the footpad of the mice, evidence of infection in the lumber dorsal root ganglia (DRG) via detection of HSV activity in the explanted DRG samples, was observed on days 24-26 post-injection. However, the short study duration (26 days) was likely not sufficient for establishment of a latent infection, thus no robust conclusions regarding latent infection and reactivation of T-VEC can be made from these data.

Pregnant mice were i.v. injected on GDs 6, 9, 12, and 15 (i.e., during organogenesis) with T-VEC at dose levels up 10-fold higher than the highest proposed clinical dose level. No adverse effects on embryo-fetal viability and development occurred. However, expressed human GM-CSF is not bioactive in mice, thus the relevancy of these results to humans as they relate to this expressed protein is unclear. Viral DNA was detected in the pooled blood sample from one litter at the highest tested dose level.

Key Words/Terms: Talimogene laherparepvec, T-VEC, Imlygic®, OncoVEX^{GM-CSF}, OncoVEX$^{mouseM-CSF}$, oncolytic HSV immunotherapy, melanoma, oncolytic HSV-1 (oHSV)